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(57) Abstract

The invention refers to a method for diagnosing the presence of infection of papilloma virus (PV) and of papilloma virus (PV) carrying tumours, especially cervix cancer and condyloma, by the detection of virus specific antigen-antibody complexes in immunossay.

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SYNTHETIC PEPTIDES OF HUMAN PAPILLOMAVIRUSES 1, 5, 6, 8, 11, 16, 18, 31, 33 AND 56, USEFUL IN IMMUNOASSAY FOR DIAGNOSTIC PURPOSES

The invention refers to a method for diagnosing the infection of human papillomavirus (HPV) and of papillomavirus (PV) carrying tumours, especially cervix cancer and condyloma, by the detection of virus specific antigen-antibody complexes in immunoassay. Several new immunoreactive antigens based on the HPV-types 1, 5, 6, 8, 11, 16, 18, 31, 33 and 56, useful for such diagnostics, have been produced by chemical means. Their use and structure is described.

Human papillomavirus is a common virus family which causes proliferative diseases in an infected epithelium. Different types of HPV cause different diseases and appear at different sites in the body. Several types, in particular type 6, 11, 16, 18, 31, 33, 35 and 52, infect the genital region. The HPV types 6 and 11 mainly cause pointed genital warts, known as condyloma acuminata. The types 16, 18, 31, 33, 35, 39, 51 and 55 cause on the other hand mainly flat, almost invisible lesions which are called cervical intraepithelial neoplasia (CIN). These CIN-lesions can develop further to cervix cancer even if comparatively seldom. More than 90 % of all cervix cancers carry some type of HPV. HPV 16 alone is found in about 60 % of all cervical tumours. HPV 1 causes benign skin tumours, verruca vulgaris or common wart. HPV 5 and HPV 8 cause malign squamous cell carcinomas in the skin, above all as a part of a state of illness with multiple warts which are called epid rmodysplasia verruciformis (EV).

All HPV genoms have at l ast 8 r gions which are supposed to code for a protein and are called open reading

frames (ORF).These are numbered E1 to E7, L1 and L2. By way of introduction, the reading frames E1, E2, E4, E5, E6 and E7 have been studied in connection with the present invention. Fortyone peptides in all have then been synthesized on the basis of the amino acid sequences for the proteins from E2, E4, E7, L1 or L2 of HPV 1, 5, 6, 8, 11, 16, 18, 31 and 33. The selection of peptide sequences was based on the assumption that an immunoreactive region might be situated in the same relative region of a protein from different HPV types. In this connection, the above described knowledge about the immunoreactive regions of HPV 16 and other known immunoreactive regions within E2, E4, E7, L1 and L2 of HPV 16 were utilized (J. Dillner, L. Dillner, WO 90/04790, Dillner et al., Int. J. Cancer, 45, 529-535, 1990; Dillner, Int. J. Cancer, 46, 703-711, 1990). It is not at all obvious that immunoreactive regions of the other HPV viruses should be located within the same relative regions, and as can be seen below, the successful discovery of immunoreactive regions have been very different for different peptides and different HPV types. Immunoreactive antigens of HPV 16 are known within the reading frames E1, E2, and E6 (J. Dillner et al., Proceedings of the National Academy of Sciences USA, 86, 3838-3841 (1989); J. Dillner, EP,A2 344 940). Additional peptides which likewise originate from HPV 16, reading frames E1, E2, E4, E6 and E7, have been described (G.K. Schoolnik, EP,A2 257 754). Moreover, these have been selected with respect to predicted secondary structure and hydrophilicity. However, these antigens differ from those claimed here.

Schoolnik et al. (EP, A2 257 754) have used a computer algorithm based on hydrophilicity and predicted secondary structur to select peptide sequ nces for synthesis. W. D. Lancaster pr s nted (in a lecture at the 7th International Papillomavirus meeting, Sophia Antopolis, France, May 1988)

the use of a computer algorithm to select sequences in the L1 protein of BPV-1 and BPV-2 that were either poorly conserved or exhibited homology between these BPV types. It must be emphasized that in no instance was any type of computer algorithm or other previously described methods used for peptide selection in the present invention.

The reading frame E7 of HPV 16 is 84 amino acids long and has been synthesized as a 84 amino acids long peptide (Reeves et al., Lancet, i, 551-552, 1990) and as a fusion protein produced in bacteria (Jochmus-Kudielka et al., J. Nat. Cancer Inst., 81, 1698-1704, 1989). The costs for producing such long peptides are, however, very high and it is in addition technicallly difficult to accomplish and the shorter E7 peptides described here are consequently a considerable improvement.

An object of the invention is to provide a method of detecting especially cervix cancer in a cheaper and more easy way. Furthermore, it was intended to find peptides based on ORF E7, which should be more immunoreactive and at the same time considerbly shorter and thus cheaper and more easy to produce than the earlier described 84 amino acid long peptides. The objec has been further developed to find synthetic peptides based on other medically important papillomaviruses than the earlier studied HPV 16. In the present invention we describe immunoreactive peptides from HPV 1, 5, 6, 8, 11, 16, 18, 31, 33 and 56.

In order to achieve said purpose the method of the invention has obtained the characterizing features of claim 1.

The invention will now be further explained below with reference to the accompanying drawings, in which

FIG 1 shows the IgA reactivity of 30 sera from patients with cervix canc r against 42 overlapping 20 amino acid peptides corresponding to the total

sequence of human papillomavirus type 16 (HPV 16), ORF E1,

FIG 2 shows the IgG reactivity of 30 sera from patients with cervix cancer against 42 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E1,

FIG 3 shows the IgM reactivity of 30 sera from patients with cervix cancer against 42 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E1,

FIG 4 shows the IgA reactivity of 30 sera from patients with cervix cancer against 24 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E2,

FIG 5 shows the IgG reactivity of 30 sera from patients with cervix cancer against 24 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E2,

FIG 6 shows the IgM reactivity of 30 sera from patients with cervix cancer against 24 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E2,

FIG 7 shows the IgA reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E4,

FIG 8 shows the IgG reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptid s corresponding to the total

sequence of human papillomavirus type 16 (HPV 16), ORF E4,

FIG 9 shows the IgM reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E4,

FIG 10 shows the IgA reactivity of 30 sera from patients with cervix cancer against 5 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E5,

FIG 11 shows the IgG reactivity of 30 sera from patients with cervix cancer against 5 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E5,

FIG 12 shows the IgM reactivity of 30 sera from patients with cervix cancer against 5 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E5,

FIG 13 shows the IgA reactivity of 30 sera from patients with cervix cancer against 10 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E6,

FIG 14 shows the IgG reactivity of 30 sera from patients with cervix cancer against 10 overlapping 20 amino acid peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E6,

FIG 15 shows the IgM reactivity of 30 sera from patients with cervix cancer against 10 overlapping 20 amino acid peptides corresponding to th total

sequence of human papillomavirus type 16 (HPV 16), ORF E6,

FIG 16 shows the IgA reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E7,

FIG 17 shows the IgG reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E7,

FIG 18 shows the IgM reactivity of 30 sera from patients with cervix cancer against 6 overlapping 20 aminosyrors peptides corresponding to the total sequence of human papillomavirus type 16 (HPV 16), ORF E7,

FIG 19 shows a comparison between the immunoreactivities of 30 sera from patients with cervix cancer and 60 sera from healthy persons against 4 of the examined peptides,

FIG 20 shows the mapping of papillomavirus group-specific epitopes, and

FIG 21 shows the generation of group specific antisera by immunization with synthetic peptides.

Peptide synthesis

The polypeptides were produced synthetically and the the following formula are stated for denoting the amino acids in the synthetic peptides.

In Table 1 below the symbols indicate amin acids according to the following:

SYMBOL	AMINO ACID
Y	L-tyrosine
G	glycine
F	L-phenylalanine
M	L-methionine
A	L-alanine
S	L-serine
I	L-isoleucine
L	L-leucine
T	L-threonine
V	L-valine
P	L-proline
K	L-lysine
H	L-histidine
Q	L-glutamine
E	L-glutamic acid
W	L-tryptophan
R	L-arginine
D	L-aspartic acid
N	L-asparagine
C	L-cysteine

The different amino acids used for peptide synthesis had beeen deduced from the nucelotide sequences of the open reading frames L1, L2, E2, E4 and E7 of the HPV types 1, 5, 6, 8, 11, 16, 18, 31 and 33. The peptide synthesis was performed with t-Boc amino acids (Bachyem, Bubendorf, Schweiz) and a p-methylbenzhydrylamine resin (Fluka, Buchs, Schweiz) according to an earlier published method (Houghten, Proceedings of the National Academy of Sciences USA, 82, 5131-5153 1985). The protecting groups of formyl-tryptophan and methionine sulphoxide were removed by treatment with 25 % hydrofluoric acid and the peptides were th n d tached from the resin by liquid hydroflu ric acid. Sinc all peptid s were synth siz d on a

p-methylbenzhydrylamine resin, all peptides contain an amide group on their carboxyterminal end.

Preparation of peptide antisera

The peptides were coupled to the carrier protein KLH (Keyhole limpet hemocyanin, an oxygen transport protein from a marine gastropod, Sigma, St Louis, MO. USA) by using maleimidobenzoyl-n-hydroxysuccinimide ester (MBS) for peptides containing cysteine or glutardialdehyde for the others. In this connection, 4 mg peptide was coupled to 4 mg KLH. The reaction mixture with KLH was dialyzed against 10 mM phosphate buffer, pH 6, and 85 µl of 4 mg/ml MBS dissolved in dimethylformamide was then added and was allowed to react for 30 minutes. KLH-MBS was then separated from free MBS by gel filtation on a column of Sepahdex G-25 (Pharmacia, Upsala, Sweden) and the synthetic peptide was added and allowed to react for 15 hours at room temperature. When coupling with glutardialdehyde, KLH was dialyzed against PBS and 4 mg KLH was then mixed with 4 mg synthetic peptide. 200 µl of 25 % glutardialdehyd (Merck, Darmstadt, Germany) was added to 13 ml PBS. Then 260 µl of this diluted glutardialdehyde solution was added to the mixture of peptide and KLH and the reaction was allowed to proceed for 15 hours at room temperature. Finally, 100 mM Tris-HCl, pH 7.5, was added. When guinea pigs were immunized, 100 µg of the coupled peptide was injected subcutaneously. At the first immunization the peptide was suspended in 1 ml of Freunds complete adjuvant (Difco). Two weeks later and after another two weeks the animals were given 100 µg of coupled peptide in Freunds incomplete adjuvant (Difco) was administered. The animals were bled two weeks after the last immunization .

Sera

Sera used for immunoassays were obtained from patients with HPV 16-carrying cervix cancer and from healthy controls.

Collection of cervical secretions

A small brush (Cytobrush, Medscand, Malmö, Sweden) was rotated over the endo and exocervix and then the brush was placed in a small vial with 1 ml of phosphate buffered saline containing 5 mM ethylenedinitrilotetraacetic acid, penicilline, streptomycine and amphotericin B. The vial was mixed on a Vortex mixer and centrifuged at 5000 rpm for 10 minutes. The brush was removed and the supernatant, containing cervical secretions, was aspirated. The secretions obtained by this method were diluted 1:2 in 10 % lamb serum/phosphate buffered saline before they were used in ELISA.

IMMUNOASSAYS

ELISA

The synthetic peptides were diluted to 20 µg/ml in 10 mM carbonate buffer, pH 9.6, and kept for 15 hours at room temperature or, alternatively, for 72 hours at +4 °C in 50 µl/hole ELISA plates (Costar, Cambridge, Mass. USA). After a washing with phosphate buffered saline (PBS) containing 0.05 % Tween 20 (PBS-T), the plates were blocked with 10 % lamb serum in PBS (LS-PBS) for 60 minutes at 37 °C or, alternatively, for 4 hours at room temperature. Human sera were diluted 1:30 or, alternatively, 1:20 in LS-PBS, and added to the plate whereupon the mixture was incubated for 120 minuters at 37 °C. After 5 washes with PBS-T, a monoclonal antibody against IgA, labelled with peroxidase (Janssen, Beers , Belgien) and diluted 1:500 or, alternatively, 1:200 in LS-PBS, was added. After 5 washes with PBS-T, 0.4 mg/ml of 2,2,-azino-di(3-ethylb nzthiaz lin -

sulfonate) diammonium salt (ABTS) in 0,1 M citrate buffer, pH 4, containing 0.9 % hydrogen peroxide was added and the plate was incubated for 60 minutes. The absorbance of the liberated colour from the reaction was recorded at 415 nm in a spectrophotometer (Titertek, Flow). After 2 washings with PBS-T and blocking with LS-PBS as described above, a rabbit antibody against human IgG, labelled with alkaline phosphatase (Dako, Copenhagen, Denmark) and diluted 1:1000 in LS-PBS, was added and the mixture was incubated for 120 minutes at 37 ^{o}C. After 5 washings with PBS-T, 1 mg/ml of para-nitrophenylphosphate in 0.1 M diethanolamine buffer, pH 9,6, containing 1 mM $MgCl_2$ was added and the mixture was incubated for 90 minutes at room temperature. The absorbance of the liberated colour from the reaction was recorded at 405 nm in a spectrophotometer (Titertek, Flow). The plates were then washed twice with PBS-T, blocked with LS-PBS as described above and a goat antibody against human IgM, labelled with glucose oxidase (Sera-lab, England) and diluted 1:800 in LS-PBS, was then added and the mixture was incubated for 120 minutes at 37 ^{o}C. After 5 washings with PBS-T, 0.36 mg/ml of ABTS, 2.4 % glukos and 8 ig/ml peroxidase in 0.1 M phosphate buffer, pH 6.0, was added. The absorbance of the liberated colour from the reaction was recorded after 60 minutes at 405 nm in a spectrophotometer (Titertek, Flow). In every test 30 sera were allowed to react with the earlier described reactive peptide HKSAIVTLTYDSEWQRDQC as an internal standard and the absorbances were adjusted compared to the internal standard in order to eliminate variations in the results between different experiments.

Immunofluorescence

Cells of an established cell line of mouse fibroblasts, which had been transfected with HPV type 16, and, as a control, cells of the same cell line, which did

not harbour HPV 16 (NIH 3t3), were allowed to grow on a glass slide and were then fixed in 50 % acetone/50 % methanol at -20 °C for 10 minutes. After blocking of the slides with 10 % goat serum in PBS (GS-PBS) for one hour, guinea pig antisera against the different peptides, diluted 1:4 in GS-PBS, were added and the mixture was incubated for 15 hours at room temperature. The slides were immersed 60 times in PBS, 15 µg/ml of goat anti-guinea pig IgG, labelled with biotin (Vector, Burlingame,Ca. USA) in GS-PBS, was added and the mixture was incubated for 45 minutes at room temperature. After 60 immersions in PBS, Avidin labelled with fluorescein isothiocyanate (Dako, Köpenhamn, Danmark) at a concentration of 10 µg/ml in a solution of 300 mM NaCl and 10 mM Tris-HCl was added and the mixture was incubated for 30 minutes at room temperature. After 60 immersions in PBS, the specimens were mounted in 50 % glycerol and the fluorescent reactivity was determined in a fluorescence microscope (Leitz, Wetzlar, Tyskland).

Immunohistocytochemistry

Four mikron thick sections of formalin fixated and paraffin imbedded preparations of cervix tissue, infected with HPV 16, HPV 6 or HPV 11, on glass slides were immersed in xylene, absolute ethanol, 95 % ethanol, 80 % ethanol, 50 % ethanol and, finally, PBS. Peroxidase activity was extincted by an immersion for 15 minutes in 3 % hydrogen peroxide in PBS and the slides were then blocked with 10 % goat serum in PBS (GS-PBS) for one hour. Guinea pig antisera against the different peptides, diluted 1:100 and 1:200 in GS-PBS, were added and allowed to react for 15 hours at room temperature. After 60 immersions in PBS, 15 µg/ml of goat-anti guinea pig-IgG, labelled with biotin (Vector, Burlingam ,Ca. USA) in GS-PBS, was added and th mixtur was incubated for 45 minut s at r om temperature. After 60 immersions in PBS, Avidin labelled with peroxidase

(Vector, Burlingame,Ca. USA) in PBS was added and the mixture was incubated for 30 minutes. After 60 immersions in PBS, the slides were immersed for 30 minutes in a solution of 200 ml 0.1 M acetate buffer, pH 5, containing 50 mg aminoethyl carbazole, 4 ml dimethyl formamide and 80 µl 30 % hydrogen peroxide. After 20 immersions in PBS the slides were immersed for 10 seconds in Mayer's haematoxylin and then again immersed 60 times in PBS. The specimens were then mounted with 50 % glycerol and examined under a light microscope (Leitz, Wetzlar, Germany).

Results

All peptides were tested in ELISA for reactivity with IgA, IgG or IgM antibodies in human sera. Peptides from HPV 16, reading frame E1, E2, E4, E5, E6 and E7 were all tested against a panel of 30 sera from patients with HPV-carrying cervix cancer. IgA reactivity against E1 peptides could first of all be detected with peptides from the carboxyterminal part of E1, FIG 1. The reactivity was comparatively low with the peptide E1:33 as the only peptide which was reactive with a majority of the sera.

Only low IgG reactivity was obtained with a minority of the sera, FIG 2. Even the most IgG reactive peptide, E1:39, did only react with 20 % of the sera. Some IgM reactivity was also found with peptides in the carboxy-terminal region of E1, FIG 3, and two peptides, E1:6 and E1:10, in the aminoterminal region of E1. The reading frame E2 was the most reactive of all HPV reading frames. 11 out of 24 peptides were IgA reactive with 25 % or more of the sera from patients with cervix cancer. The three most reactive peptides, E2:9, E2:13 and E2:17, were IgA reactive with 70 % or more of the sera from patients with cervix cancer, FIG 4. The IgG reactivity was somewhat lesser but two peptids, E2:9 and E2:13, were reactive with as much as 77 and 73 %, respectively, of the cervix cancer sera,

FIG 5. One of the peptides which were very reactive with IgA, peptide E2:17, was not reactive with IgG to any appreciable extent. The IgM reactive peptides from E2 differed to a large extent from the IgA and IgG reactive peptides, FIG 6. For example, the IgA reactive peptides E2:9 and E2:13 were not appriciably IgM reactive, while the peptide E2:17 reacted with IgA and IgM but not with IgG.

The reading frame E4 contained a main epitope for IgA antibodies, peptide E4:4, FIG 7. This peptide was also the most important E4 epitope for IgG and IgM antibodies, FIG 8 and 9. IgG reactivity was also found with peptide E4:6 at the carboxyterminal end of E4.

The reading frame E5 contained only one epitope worth mentioning, E5:1, at the aminoterminal end of E5, which had some reactivity with IgA, IgG as well as IgM, FIG 10, 11 and 12.

Nor was the reading frame E6 particularly imunnoreactive. Comparatively low IgA and IgG reactivities were noted, FIG 13 and 14. However, several peptides were IgM reactive with the peptide E6:6 as the most reactive (47 % of the sera from patients with cervix cancer), FIG 15.

The immunoreactivity of the E7 peptides were unusual. For IgG as well as IgA, a small portion (10-20 %) of the sera exhibited extremely poternt reactivity, FIG 16 and 17. These comparatively unusual but strong reactivities were obtained for more peptides along the total E7, FIG 16 and 17. The IgM reactivity against the E7 peptides were dominated by peptide E7:4, FIG 18.

Ten of the most reactive peptides were also tested with a panel of 60 sera from healthy persons who then ought to have a smaller portion of and neither as active HPV 16 infections as the cervix cancer group. The absorbance values from ELISA for the two groups were compared with r spect t statistically significant differences with aMann-Whitney test. Most of thes 10 peptid s w re a also

little reactive with the control sera as shown in Table 2 below.

TABLE 2

Peptide	IgA			IgG			IgM		
	>0.1[1]		p-value[2]	>0.1		p-value	>0.1		p-valu
	SCC	Co		SCC	Co		SCC	Co	
EI:33	63 %	37 %	<0.02	3 %	2 %	NS	40 %	20 %	NS
E1:39	43 %	18 %	NS	20 %	0 %	<0.0001	30 %	8 %	<0.0001
E2:9	70 %	62 %	<0.01	76 %	82 %	NS	13 %	12 %	NS
E2:13	87 %	68 %	<0.0001	73 %	47 %	<0.0005	17 %	20 %	NS
E2:17	70 %	55 %	<0.01	7 %	5 %	NS	97 %	78 %	NS
E2:19	30 %	20 %	NS	3 %	2 %	NS	80 %	80 %	NS
E4:4	67 %	30 %	<0.0001	37 %	28 %	NS	37 %	37 %	NS
E6:6	23 %	13 %	NS	3 %	2 %	NS	47 %	28 %	<0.02
E7:1	23 %	2 %	<0.0002	20 %	8 %	<0.03	13 %	2 %	NS
E7:4	30 %	2 %	<0.0002	3 %	2 %	NS	80 %	75 %	NS

1) Per cent of sera which gives an absorbance value from ELISA of more than 0.1 in the group with cervical carcinoma (SCC) or in the control group (Co).

2) The numerals indicate the p-values for an significant increase in the absorbances in the group with cervical carcinoma. Notice that the p-values are not based on the percentage of positive sera but on a non-parametric method (Mann-Whitmey-test) of the actual absorbances.

NS = Not significant

In several cases, however, very steep increases of antibody titers against these peptides were obtained with patients with cervix cancer compared with the healthy persons as is evident from Table 2 and FIG 19. The IgG reactivities against the peptides E2:13, E7:1 and E1:39 as well as the IgA reactivities against E2:9, E2:13, E2:17, E7:1, E7:4 and E4:4 should especially be mentioned. According to Table 2, the IgM reactivities were tumour associated only in a couple of cases.

Ninety-four peptides (all from E1, E2, E4, E5, E6 and E7) were also coupled to a carrier protein and used for producing anti-peptide antibodies in guinea pigs by hyperimmunization. The production of anti-peptide antibodies by hyperimmunization was verified by ELISA with a peptide which had not been coupled. All antisera were tested with respect to fluorescence against the cell lines NIH3T3 and NIH3T3/HPV 16. Several sera exhibited immunofluorescence. The antiserum against E6:1 was that serum which gave the most substantial immunofluorescence in the HPV positive cell while no fluorescence was obtained in the HPV negative cell (not shown).

All peptides were tested in ELISA with respect to reactivity with IgA, IgG or IgM antibodies in human sera. The HPV types 1, 5 and 8 infect the skin, HPV 1 gives common benign warts while HPV 5 and 8 are asssociated with malign squamous cell carcinomas in the skin, and since it is well known that warts are very frequent with children the peptides for these three types were tested with a panel of 30 sera. The first ten sera are from children (2-8 years old), the following from patients with cervix cancer, where the HPV type in the tumour has not been determined, and the last ten sera are from patients with CIN where likewise the HPV type has not been determined. Th results ar given as the obtained optical densities at 415 nm multipli d with

1000. Values below 100 are not considered as positive but are included for the sake of completeness.

Peptide IGSARMLVKFIDEAQREKC, HPV 1, protein E2.

IgA: 0, 541, 918, 186, 52, 0, 48, 0, 166, 37, 5, 0, 0, 0, 0, 3, 0, 36, 51, 0, 0, 0, 0, 11, 27, 48, 17, 113, 0, 0.

IgG: 185, 106, 117, 122, 183, 45, 276, 167, 94, 94, 204, 44, 80, 37, 72, 85, 176, 44, 49, 32, 85, 180, 55, 25, 51, 48, 73, 347, 40, 0.

IgM: 102, 88, 206, 70, 87, 49, 37, 1335, 936, 151, 146, 64, 0, 39, 0, 0, 1, 0, 33, 0, 58, 33, 55, 18, 14, 46, 14, 204, 13, 28.

Peptide YDNNPDNQTRHTIWNHVYYQ, HPV 1, E2.

IgA: 0, 28, 13, 0, 30, 3, 0, 0, 38, 9, 0, 24, 45, 0, 4, 33, 0, 84, 77, 0, 0, 16, 0, 31, 90, 114, 98, 67, 52, 0.

IgG: 150, 189, 165, 144, 249, 57, 304, 206, 109, 74, 305, 98, 176, 143, 227, 172, 386, 141, 86, 107, 182, 379, 116, 92, 125, 134, 164, 271, 138, 74.

IgM: 31, 17, 9, 105, 13, 16, 6, 311, 601, 139, 56, 93, 57, 8, 54, 38, 17, 14, 42, 24, 42, 26, 14, 23, 10, 11, 32, 17, 0, 0.

Peptide LGSSLAAKCPEQAPPEPQTDPY, HPV 1, L1.

IgA: 78, 93, 209, 38, 89, 40, 249, 56, 79, 92, 13, 200, 49, 35, 15, 50, 33, 100, 115, 0, 37, 678, 152, 53, 92, 396, 202, 64, 86, 0.

IgG: 219, 330, 209, 169, 217, 87, 355, 339, 169, 211, 211, 163, 218, 183, 316, 179, 367, 177, 131, 166, 165, 387, 98, 88, 102, 207, 162, 174, 68, 38.

IgM: 272, 56, 164, 326, 93, 167, 33, 686, 458, 415, 0, 109, 11, 13, 110, 0, 26, 84, 51, 75, 36, 27, 84, 64, 16, 54, 54, 7, 11, 148.

Peptide DIPLVELNLGLETDTSSVVQ, HPV 1, L2.
IgA: 55, 0, 22, 0, 26, 48, 129, 0, 0, 0, 8, 0, 0, 0, 2, 50, 29, 0, 51, 0, 79, 68, 237, 13, 24, 81, 67, 23, 27, 0.
IgG: 223, 433, 276, 220, 264, 131, 347, 245, 128, 138, 315, 85, 184, 247, 247, 200, 479, 146, 83, 127, 370, 641, 259, 93, 104, 178, 95, 229, 109, 61.
IgM: 11, 0, 0, 50, 0, 0, 0, 95, 61, 0, 0, 0, 8, 0, 0, 0, 0, 0, 0, 26, 0, 0, 0, 0, 0, 0, 0, 0, 0.

Peptide LGRPRMLISFSSYTQRRDC, HPV 5, E2.
IgA: 0, 0, 0, 0, 9, 0, 0, 0, 0, 0, 0, 0, 0, 0, 31, 0, 0, 0, 68, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
IgG: 108, 84, 129, 72, 62, 31, 316, 147, 70, 74, 130, 44, 82, 47, 69, 72, 125, 69, 78, 48, 40, 141, 33, 32, 50, 43, 62, 107, 66, 3.
IgM: 0, 0, 28, 0, 0, 0, 32, 0, 0, 0, 23, 48, 5, 3, 0, 11, 12, 1, 48, 0, 46, 49, 6, 8, 39, 11, 27, 24, 0, 6.

Peptide LGRSRMLILFTSAGQRKDC, HPV 8, E2.
IgA: 0, 0, 195, 0, 48, 134, 427, 0, 0, 70, 503, 152, 1117, 401, 14, 843, 456, 577, 1581, 181, 93, 1396, 0, 15, 47, 213, 365, 415, 4, 0.
IgG: 115, 138, 259, 71, 80, 22, 192, 98, 87, 45, 623, 60, 194, 175, 259, 157, 404, 134, 125, 132, 165, 869, 40, 20, 35, 28, 57, 484, 21, 0.
IgM: 88, 72, 75, 0, 103, 48, 47, 0, 0, 0, 125, 94, 9, 89, 64, 53, 49, 0, 23, 36, 125, 54, 21, 57, 66, 35, 34, 12, 0, 12.

It is notable that the sera number 8 and 9 are considerably IgM positive with 3 out of 4 HPV 1 peptides and that the peptide from HPV 8 was considerably IgA positive for patients with cervix cancer but not for childr n.

HPV 6 and HPV 11 are two closely related viruses which cause pointed genital warts (condyloma) and rar ly becomes

malignant. In some rare cases can, however, HPV 6 and 11 also be associated with CIN and cervix cancer. The test panel for HPV 6 consisted of 2 sera from patients with condyloma and known HPV 6 infection, 8 sera from patients with condyloma, 4 sera from patients with cervix cancer, 6 sera from patients with condyloma, 5 sera from patients with CIN and finally further 5 sera from patients with condyloma. The test panel for HPV 11 consisted of one serum from a patient with condyloma and HPV 11, 9 sera from patients with condyloma, 4 sera from patients with cervix cancer, 6 sera from patients with condyloma, 5 sera from patients with CIN, 4 sera from patients with condyloma and finally one serum from a patient with cervix cancer.

Peptide FDGCANNTMDYVVWTDVYVQ, HPV 6, E2.
IgA: 318, 0, 340, 607, 422, 242, 352, 421, 193, 112, 160, 340, 198, 171, 259, 66, 129, 101, 296, 229, 140, 206, 569, 253, 292, 119, 150, 92, 72, 236.
IgG: 207, 229, 517, 638, 259, 430, 224, 407, 357, 257, 482, 120, 356, 333, 404, 143, 11, 276, 172, 123, 382, 674, 141, 130, 186, 304, 145, 276, 164, 241.
IgM: 0, 0, 6, 54, 4, 1, 8, 0, 25, 0, 17, 14, 22, 5, 13, 0, 0, 5, 3, 26, 50, 62, 2, 80, 23, 2, 0, 17, 0, 20.

Peptide RLGNEHEESNSPLATPCVWP, HPV 6, E4.
IgA: 33, 0, 61, 690, 63, 29, 99, 152, 132, 32, 0, 0, 0, 2, 80, 35, 37, 93, 56, 43, 0, 0, 0, 0, 52, 225, 48, 396, 46, 42.
IgG:0, 0, 0, 0, 0, 0, 363, 0, 11, 0, 0, 16, 0, 0, 0, 0, 0, 7, 0, 0, 0,3, 7, 0, 0, 28, 0, 0, 0, 0.
IgM: 0, 0, 0, 131, 0, 0, 0, 0, 18, 41, 0, 45, 49, 87, 57, 10, 0, 9, 42, 89, 135, 49, 0, 49, 0, 20, 0, 59, 126, 27.

Peptide PLDTFVVSSSDSGPTSSTPV, HPV 6, L2.
IgA: 17, 1699, 10, 770, 14, 867, 20, 0, 255, 5, 511, 2682, 72, 4, 286, 108, 321, 285, 66, 2750, 159, 2604, 0, 46, 0, 2834, 48, 24, 88, 122.
IgG: 290, 2944, 2438, 955, 222, 2972, 436, 369, 440, 165, 528, 2651, 1301, 323, 953, 690, 93, 869, 157, 2961, 920, 2898, 351, 241, 197, 2952, 254, 552, 1550, 317.
IgM: 0, 0, 0, 122, 0, 0, 0, 0, 95, 0, 7, 0, 0, 0, 44, 8, 0, 0, 2, 0, 25, 0, 0, 0, 0, 0, 34, 0, 0, 0.

Peptide FDGCEDNVMEYVVWTHIYLQ, HPV 11, E2.
IgA: 178, 173, 474, 381, 229, 270, 322, 236, 122, 227, 52, 252, 170, 89, 75, 105, 181, 239, 191, 145, 190, 134, 440, 176, 151, 134, 92, 113, 169, 0.
IgG: 140, 613, 634, 346, 314, 254, 351, 414, 207, 519, 401, 153, 383, 374, 148, 44, 368, 278, 258, 291, 387, 633, 250, 125, 177, 215, 284, 241, 251, 311.
IgM: 0, 0, 246, 0, 0, 0, 0, 7, 0, 0, 1, 17, 8, 0, 0, 0, 0, 19, 0, 0, 13, 22, 0, 0, 0, 0, 0, 0, 0, 54.

Peptide RRRLGSEHVDRPLTTPCVWP, HPV 11, E4.
IgA: 0, 26, 0, 0, 0, 0, 232, 22, 0, 31, 0, 0, 0, 0, 0, 0, 0, 64, 0, 41, 0, 0, 0, 5, 0, 0, 11, 0, 0, 67.
IgG: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
IgM: 10, 0, 17, 0, 0, 4, 191, 0, 0, 88, 13, 45, 0, 0, 4, 0, 0, 6, 0, 0, 40, 32, 0, 0, 3, 0, 83, 0, 0, 0.

Peptide QSQAITCQKPTPEKEKQDPYK, HPV 11, L1.
IgA: 168, 48, 143, 137, 396, 123, 282, 229, 79, 511, 61, 329, 289, 554, 247, 62, 68, 235, 69, 76, 0, 625, 0, 786, 97, 0, 49, 129, 240, 0.
IgG: 46, 21, 122, 71, 148, 78, 46, 127, 47, 149, 150, 60, 386, 115, 78, 8, 154, 141, 100, 237, 200, 447, 115, 113, 126, 151, 204, 215, 219, 285.

IgM: 92, 0, 139, 21, 0, 0, 0, 319, 5, 248, 134, 759, 70, 46, 11, 0, 107, 285, 141, 591, 129, 172, 254, 27, 47, 3, 205, 375, 124, 262.

Peptide PLDTFVVSSSDSGPTSSTPL, HPV 11, L2.
IgA: 0, 0, 83, 0, 132, 0, 0, 41, 0, 0, 0, 438, 9, 0, 51, 29, 144, 54, 300, 2621, 0, 1738, 0, 31, 0, 21, 17, 0, 36, 0.
IgG: 143, 2288, 338, 127, 431, 245, 218, 285, 91, 0, 428, 2712, 541, 217, 206, 34, 495, 178, 1167, 128, 155, 881, 201, 135, 126, 187, 283, 159, 197, 201.
IgM: 0, 0, 124, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 26, 0, 0, 0, 1, 0, 0, 18, 0, 0, 0, 0, 76, 0, 0, 0, 56.

HPV 16 is that HPV type which is most commonly found with cervix cancer and CIN. The test panel for HPV 16 consisted of 10 sera from patients with cervix cancer and known HPV 16 infection, 10 sera from patients with cervix cancer and finally 10 sera from patients with CIN.

Peptide HGDTPTLHEYMLDLQPETTDLYCYEQLNDS, HPV 16, E7.
IgA: 183, 0, 0, 0, 3, 4, 80, 30, 0, 16, 18, 20, 0, 0, 0, 0, 0, 0, 34, 0, 74, 0, 188, 0, 0, 0, 12, 0, 0, 0.
IgG: 1283, 361, 0, 0, 0, 0, 93, 0, 0, 0, 18, 0, 8, 8, 0, 7, 0, 0, 0, 0, 0, 8, 2, 0, 0, 0, 0, 0, 0, 0.
IgM: 0, 25, 47, 13, 37, 40, 107, 44, 17, 38, 19, 39, 35, 30, 0, 24, 33, 11, 137, 17, 38, 43, 0, 5, 33, 10, 46, 15, 0, 14.

Peptide QAEPDRAHYNIVTFCCKCDSTLRLCVQSTH, HPV 16, E7.
IgA: 0, 43, 79, 142, 91, 162, 365, 78, 133, 266, 278, 491, 270, 184, 13, 288, 220, 106, 504, 0, 0, 215, 946, 536, 102, 149, 761, 243, 472, 0.
IgG: 20, 0, 6, 0, 7, 24, 36, 0, 8 ,7, 108, 25, 58, 39, 53, 34, 70, 30, 25, 0, 18, 84, 4, 19, 32, 13, 129, 89, 7, 0.

IgM: 155, 163, 148, 298, 254, 698, 261, 81, 289, 144, 1831, 600, 269, 374, 364, 265, 137, 497, 308, 141, 166, 161, 354, 395, 256, 143, 612, 236, 1037, 163.

Peptide CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL, HPV 16, E7.
IgA: 351, 188, 277, 99, 334, 427, 954, 375, 344, 833, 459, 634, 707, 615, 17, 834, 518, 967, 1651, 566, 255, 607, 1150, 485, 519, 426, 380, 335, 415, 107.
IgG: 33, 103, 0, 0, 17, 23, 20, 0, 6, 10, 190, 20, 108, 69, 174, 40, 112, 10, 0, 0, 55, 91, 0, 14, 20, 0, 0, 23, 0, 0.
IgM: 0, 26, 22, 33, 33, 341, 23, 2, 3, 45, 83, 20, 15, 188, 100, 26, 24, 0, 325, 13, 61, 48, 0, 52, 247, 3, 54, 282, 4, 0.

Peptide VQSTHVDIRTLEDLLMGTLGIVCPICSQKP, HPV 16, E7.
IgA: 14, 66, 68, 19, 69, 75, 159, 77, 41, 117, 63, 133, 54, 0, 17, 104, 17, 43, 112, 0, 0, 91, 1, 50, 89, 72, 35, 30, 78, 0.
IgG: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
IgM: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 118, 2, 0, 0, 0, 0, 0, 0, 4, 0, 7, 0, 0, 0, 0, 0, 0, 0, 0, 0.

New peptide from HPV 16, L1

The nucleotide sequence of the L1 open reading frame of HPV 16 was recently determined a second time (Parton, Nucleic Acids Research, 18, 3631 (1990)). It was found that the original nucleotide sequence contained two errors that changed the deduced amino acid sequence at two positions close to each other in the carboxyterminal part of L1. To investigate whether these corrections were important, a peptide covering the region of these 2 errors and a peptide from the same region but with the c rrected sequenc wer synthesized. They were test d in IgA ELISA with a pan l of

30 sera from patients with HPV-carrying CIN or cervical cancer with the following results given as absorbances:

Peptide VTQAIACQKHTPPAPKEDDPL (old, incorrect sequence): .110, 1.854, 0, 0, 0, .571, .195, .123, 0, 0, 0, 0, 0, 0, 0, 0,0, 0, 0, .343, 0, 0, 0, 0, 0, 0, 0, 0, 0, .383.

Peptide VTSQAIACQKHTPPAPKEDPL (corrected sequence): .124, 2.903, 0, 0, 0, 1.012, .289, .228, 0, 0, 0, .374, 0, 0, 0,0, 0, .125, 0, .610, 0, 0, .162, 0, 0, 0, .197, 0, 0, .632.

Results with an IgG ELISA gave a similar tendency (not shown).

The old peptide is partially overlapped by the peptides number 30:GGTLEDTYRFVTQAIACQKH and number 31:ACQKHTPPAPKEDDPLKKYT, which were described in our previous patent application. While peptide 30 was not significantly immunoreactive, peptide 31 was reactive. Comparison of the immunoreactivity of peptide 31 with peptide VTQAIACQKHTPPAPKEDDPL showed that peptide VTQAIACQKHTPPAPKEDDPL represented a slight improvement over peptide 31 (not shown).

The peptide E2:9 in FIG 4, 5 and 6 had the sequence FDGDICNTMHYTNWTHIYIC. In order to see if the immunoreactivity of this peptide could be improved, we synthesized two analogs of this peptide that were moved 2 amino acids compared to the original peptide, either in the N-terminal (peptide E2:9(N): VQFDGDICNTMHYTNWTHIY) or in the C-terminal direction (peptide E2:9(C): GDICNTMHYTNWTHIYICEE). They were then tested in an IgA ELISA with a panel of 30 sera from patients with CIN (The number of IgA-positive sera in this panel were lesser than in that panel with cervical cancer sera which was used in FIG 4, 5 and 6).

Peptide E2:9:
0, 0, 0, .105, 0, 0, 0, 0, 0, 0, 0, 0, 0, .199, 0, 0, 0, 0, 0, 0,.124, .102, .164, .126, 0, 0, 0, 0, 0, 0.

Peptide E2:9(C):
.222, 0, .253, .136, 0, 0, .159, 0, 0, 0, 0, 0, 0, .172, 0, 0, 0,0, 0, 0, .172, .210, .233, .161, 0, 0, 0, .134, .104, .155.

As can be seen, all the sera that were positive for the original peptide were also positive for the improved peptide. In all cases but one (serum number 14, .199 versus .172) the immunoreactivity was improved when peptide E2:9(C) was used. An IgG ELISA with the same sera and peptides showed that peptide E2:9(C) was an improvement also for IgG (not shown). The peptide analog E2:9 (N) gave similar results as did peptide E2:9 (not shown).

Use of peptides from L1 of HPV 16 to produce antipeptide sera that will detect all types of papillomaviruses.

In our previous patent application, peptides based on the deduced amino acid sequence of HPV 16 L1 were synthesized. The peptides were used in ELISA for detection of human antipeptide antibodies to these peptides. It was also proposed in the patent claims that these peptides could be used in the diagnosis of papillomavirus associated disease by the detection of antigen-antibody complexes in tissue. The claims also included peptides containing the same epitope and peptides containing substantial homology with the original peptide.

Group-specific detection does not necessarily mean that every single virus within the papillomavirus group should be det ctable but the d tection should be broadly reactive with genetically diverse papillomavirus types from several host species.

Thus, which ones of these HPV 16-derived peptides that were reactive with antibodies against bovine, canine and avian papillomaviruses was now tested to see which epitopes that were shared between these papillomaviruses with a very low degree of relatedness. As shown in FIG 20, peptides 16, 30, 31 and 33 from WO 90/04790 represented the main group-specific epitopes.

The prospects of producing antibodies against bovine papillomavirus (BPV) were then examined by immunization of guinea pigs with peptides 16, 30, 31 and 33. For comparison, antisera against all the other peptides were also produced. However, as shown in FIG 21, very low or no reactivity at all against BPV was found in ELISA.

Shortened versions of the original peptides 16, 30, 31 and 33 were then synthesized and used for immunization. As shown in FIG 21, the antipeptide antisera were now reactive with BPV. The most succesful peptide was peptide 16a, with the sequence VHTGFGAMDFTTLQAGGC, which gave an anti-BPV titer of 12.500 which should be compared to the anti-BPV titer of the antiserum against the original peptide 16 which was less than 100 (i e not significant).

For peptide 30b (GGTLEDTYRFGGC), an anti-BPV reaction was also obtained, which was not detectable for the original peptide.

For peptide 33a (SADLDQFPLGRKFLLGGC), the reactivity towards BPV was improved but the antiserum against the original peptide 33 also produced some anti-BPV antibodies. The antiserum to peptide 16a was then analyzed for its reactivity in immunohistocytochemical staining of slides conatining sections of human and bovine skin warts and was found to stain both types of viruses in this application in accordance with the ELISA data (not shown).

HPV 18 is a common virus associated with CIN and cervix cancer, specially adenocarcinoma. The test panel for HPV 18 consisted of 7 sera from patients with cervix

cancer and known HPV 18 infection, 2 sera from patients with CIN and known HPV 18 infection, 11 sera from patients with cervix cancer and finally 10 sera from patients with CIN.

Peptide FDGNKDNCMTYVAWDSVYYM, HPV 18, E2.
IgA: 27, 3, 0, 22, 15, 89, 74, 75, 7, 0, 0, 60, 60, 0, 0, 19, 19, 24, 83, 0, 84, 0, 393, 79, 98, 146, 0, 36, 0.
IgG: 282, 371, 222, 146, 220, 315, 442, 339, 169, 0, 581, 176, 406, 412, 498, 300, 696, 306, 192, 194, 438, 628, 279, 157, 329, 280, 233, 357, 162, 75.
IgM: 42, 55, 0, 28, 39, 30, 28, 0, 0, 0, 85, 49, 103, 25, 48, 28, 31, 18, 41, 31, 76, 76, 50, 30, 46, 22, 66, 21, 29, 0.

Peptide SLLNSYSTPPHRIPAPCPWA, HPV 18, E4.
IgA: 34, 0, 0, 0, 61, 46, 62, 0, 0, 0, 0, 17, 35, 0, 5, 0, 0, 0, 35, 0, 0, 0, 293, 0, 17, 0, 14, 32, 0, 0.
IgG: 0, 0, 0, 3, 0, 0, 0, 0, 0, 0, 0, 33, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 9, 0, 0, 0.
IgM: 24, 0, 0, 0, 10, 9, 0, 0, 1, 0, 0, 17, 36, 2, 4, 0, 20, 12, 30, 0, 33, 78, 0, 0, 62, 0, 0, 31, 0, 0.

Peptide HGPKATLQDIVLHLEPQNEIPVDLLCHEQL, HPV 18, E7.
IgA: 171, 48, 0, 21, 77, 108, 294, 119, 24, 0, 41, 271, 80, 40, 0, 121, 181, 37, 252, 48, 148, 145, 517, 156, 150, 212, 236, 17, 155, 0.
IgG: 0, 17, 0, 21, 4, 4, 4, 0, 0, 0, 23, 9, 14, 0, 0, 3, 8, 0, 0, 0, 0, 38, 0, 0, 0, 0, 0, 0, 0, 0.
IgM: 17, 38, 0, 22, 22, 31, 10, 0, 1, 0, 41, 65, 12, 8, 19, 19, 0, 31, 0, 65, 38, 0, 4, 8, 18, 23, 0, 0, 0.

P ptide PQNEIPVDLLCHEQLSDSEEENDEIDGVNH, HPV 18, E7.
IgA: 0, 0, 0, 20, 3, 0, 24, 205, 11, 0, 0, 58, 0, 0, 0, 15, 0, 0, 85, 0, 5, 0, 16, 0, 0, 35, 115, 5, 19, 0.

IgG: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 6, 3, 0, 0, 0, 0, 0, 0, 0, 0, 10, 5, 0, 18, 0, 10, 0, 0, 0.
IgM: 20, 16, 0, 3, 14, 2, 0, 0, 31, 0, 25, 60, 0, 4, 0, 13, 9, 1, 33, 0, 146, 54, 0, 4, 8, 14, 17, 70, 4, 2.

Peptide SDSEEENDEIDGVNHQHLPARRAEPQRHTM, HPV 18, E7.
IgA: 0, 0, 0, 2, 37, 67, 1410, 0, 60, 0, 0, 41, 0, 0, 18, 28, 0, 0, 55, 0, 0, 0, 0, 0, 73, 100, 80, 33, 22, 0.
IgG: 0, 0, 0, 30, 0, 0, 0, 0, 0, 0, 0, 4, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 2, 2, 3, 0, 0.
IgM: 4, 0, 0, 0, 2, 0, 0, 0, 19, 0, 0, 54, 0, 0, 0, 0, 0, 0, 51, 0, 29, 26, 0, 0, 15, 1, 6, 50, 0, 35.

Peptide QHLPARRAEPQRHTMLCMCCKCEARIELVV, HPV 18, E7.
IgA: 331, 236, 83, 7, 193, 202, 141, 0, 30, 266, 144, 627, 301, 155, 14, 440, 213, 94, 662, 0, 0, 971, 0, 0, 4, 520, 243, 506, 102, 0.
IgG: 0, 34, 0, 0, 13, 6, 0, 0, 0, 0, 14, 63, 31, 13, 8, 19, 0, 7, 7, 0, 1, 122, 0, 0, 0, 156, 16, 26, 39, 0.
IgM: 23, 143, 59, 162, 131, 143, 29, 121, 96, 136, 82, 80, 61, 130, 104, 83, 63, 99, 124, 58, 137, 22, 22, 46, 129, 146, 131, 27, 44, 121.

Peptide LCMCCKCEARIELVVESSADDLRAFQQLFL, HPV 18, E7.
IgA: 374, 28, 0, 44, 137, 205, 484, 27, 28, 71, 114, 1835, 23, 87, 10, 155, 103, 435, 360, 492, 145, 74, 339, 540, 243, 453, 225, 0, 219, 0.
IgG: 565, 606, 438, 266, 414, 546, 758, 432, 275, 468, 823, 195, 492, 687, 710, 466, 929, 346, 331, 185, 663, 952, 464, 335, 381, 399, 260, 386, 211, 100.
IgM: 26, 39, 0, 32, 40, 109, 119, 15, 66, 29, 77, 28, 0, 137, 0, 31, 20, 156, 116, 50, 135, 45, 30, 60, 68, 49, 50, 30, 92, 10.

Peptide ESSADDLRAFQQLFLNTLSFVCPWCASQQ, HPV 18, E7.
IgA: 91, 12, 0, 7, 104, 243, 140, 0, 5, 18, 0, 87, 0, 0, 0, 60, 0, 0, 151, 0, 0, 79, 0, 15, 72, 155, 121, 0, 102, 0.
IgG: 331, 376, 287, 226, 271, 304, 438, 217, 147, 216, 392, 186, 354, 280, 366, 319, 551, 229, 218, 135, 325, 520, 254, 181, 190, 129, 140, 239, 92, 39.
IgM: 55, 103, 0, 81, 152, 417, 98, 36, 52, 7, 32, 205, 36, 43, 0, 27, 53, 24, 52, 22, 151, 125, 180, 76, 139, 156, 142, 36, 352, 153.

Peptide QSVAITCQKDAAPAENKDPYD, HPV 18, L1.
IgA: 223, 0, 0, 0, 0, 45, 0, 53, 0, 0, 0, 0, 10, 0, 0, 32, 0, 0, 4, 0, 0, 0, 1, 0, 9, 0, 28, 66, 24, 0.
IgG: 226, 233, 192, 90, 126, 597, 286, 248, 138, 0, 376, 46, 135, 83, 149, 118, 320, 108, 320, 108, 66, 117, 272, 435, 108, 30, 99, 118, 94, 405, 99, 25.
IgM: 50, 9, 99, 58, 12, 0, 0, 20, 65, 0, 32, 57, 24, 0, 0, 0, 0, 0, 13, 0, 25, 48, 70, 12, 95, 0, 0, 42, 115, 29.

Peptide PLQTFASSGTGEEPISSTPL, HPV 18, L2.
IgA: 270, 124, 0, 25, 31, 176, 34, 203, 109, 11, 143, 933, 43, 13, 0, 89, 17, 0, 26, 14, 169, 2604, 33, 41, 30, 1556, 689, 453, 62, 0.
IgG: 1449, 350, 336, 143, 155, 280, 382, 410, 312, 166, 470, 2751, 990, 213, 519, 1468, 457, 255, 94, 166, 537, 2181, 332, 223, 133, 918, 1513, 420, 189, 146.
IgM: 0, 63, 0, 0, 4, 200, 0, 30, 27, 0, 41, 8, 0, 205, 61, 0, 0, 0, 0, 0, 127, 0, 0, 0, 7, 12, 55, 46, 47, 1.

HPV 31 is also a common virus in connection with CIN and is sometimes seen in connection with cervix cancer. The test panel for HPV 31 consisted of 10 sera from patients with CIN and known HPV 31 infecti n, 10 s ra from patients with cervix cancer and 10 sera from patients with CIN.

Peptide FDGDVHNTMHYTNWKFIYLC, HPV 31, E2.
IgA: 24, 0, 28, 0, 31, 16, 9, 31, 0, 106, 33, 183, 117, 210, 10, 158, 88, 101, 303, 0, 0, 82, 64, 127, 164, 197, 156, 49, 231, 0.
IgG: 24, 0, 21, 0, 19, 16, 8, 37, 3, 0, 13, 14, 33, 1, 35, 40, 72, 34, 14, 9, 28, 86, 0, 4, 0, 16, 55, 55, 26, 0.
IgM: 0, 0, 0, 0, 0, 0, 0, 24, 0, 39, 23, 63, 39, 1, 6, 35, 44, 47, 93, 35, 52, 56, 35, 45, 52, 46, 35, 60, 74, 28.

Peptide HKNAIVTLTYISTSQRDDC, HPV 31, E2.
IgA: 120, 0, 88, 46, 133, 122, 15, 87, 0, 270, 281, 336, 390, 213, 55, 486, 199, 319, 1011, 70, 6, 306, 335, 210, 1868, 318, 379, 272, 213, 0.
IgG: 296, 220, 158, 99, 117, 201, 245, 255, 264, 159, 1056, 369, 494, 310, 722, 263, 697, 342, 309, 276, 338, 538, 140, 117, 194, 166, 240, 428, 238, 94.
IgM: 104, 67, 52, 26, 44, 59, 17, 52, 0, 113, 167, 133, 74, 76, 219, 59, 39, 70, 653, 114, 241, 145, 37, 221, 2227, 50, 69, 120, 137, 35.

Peptide TSQAITCQKTAPQKPKEDPFK, HPV 31, L1.
IgA: 0, 0, 10, 0, 0, 0, 10, 4, 0, 61, 6, 64, 48, 15, 0, 0, 0, 108, 0, 0, 29, 0, 22, 16, 84, 24, 222, 17, 0.
IgG: 2, 0, 8, 1, 18, 13, 15, 33, 0, 0, 0, 9, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 78, 0, 0.
IgM: 255, 65, 319, 524, 18, 35, 9, 87, 223, 140, 15, 398, 31, 294, 139, 0, 0, 0, 39, 0, 40, 129, 104, 0, 9, 17, 49, 239, 301, 184.

Peptide PMDTFIVSTNNQNITSSTPI, HPV 31, L2.
IgA: 206, 207, 729, 121, 625, 1343, 12, 250, 91, 755, 1144, 2682, 144, 107, 4, 317, 22, 117, 108, 39, 238, 2604, 0, 51, 96, 2800, 2406, 2831, 762, 0.

IgG: 64, 255, 144, 13, 326, 116, 741, 162, 77, 259, 43, 583, 214, 12, 298, 713, 0, 76, 10, 57, 103, 473, 12, 19, 1, 709, 393, 0, 557, 126.
IgM: 29, 96, 0, 153, 0, 0, 0, 17, 131, 74, 21, 0, 0, 95, 86, 0, 0, 0, 22, 7, 121, 0, 12, 5, 10, 29, 0, 0, 8, 0.

HPV 33 is a relatively new virus which has been found to be rather common both in connection with CIN and cervix cancer. The test panel for HPV 33 consisted of 4 sera from patients with cervix cancer and known HPV 33 infection, 5 sera from patients with CIN and known HPV 33 infection, 11 sera from patients with cervix cancer and finally 10 sera from patients with CIN.

Peptide YDNDKKNTMDYTNWGEIYII, HPV 33, E2.
IgA: 53, 0, 29, 204, 67, 65, 19, 83, 210, 47, 12, 170, 77, 551, 35, 140, 146, 71, 150, 103, 210, 87, 347, 143, 180, 222, 241, 110, 171, 0.
IgG: 165, 268, 2202, 237, 240, 415, 97, 211, 333, 147, 458, 191, 323, 384, 330, 250, 714, 185, 146, 142, 459, 541, 250, 110, 124, 242, 148, 335, 149, 65.
IgM: 4, 0, 0, 0, 0, 0, 0, 0, 2, 155, 7, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 24, 0, 0, 0, 0, 0, 7, 0, 0.

Peptide PQTPPSPLQSCSVQTPPWTI, HPV 33, E4.
IgA: 92, 0, 28, 14, 0, 22, 7, 38, 127, 62, 73, 200, 74, 138, 44, 227, 49, 61, 283, 0, 41, 121, 69, 88, 145, 68, 37, 81, 84, 0.
IgG: 6, 5, 0, 1, 19, 0, 0, 0, 0, 0, 0, 27, 0, 15, 0, 0, 0, 20, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0.
IgM: 355, 66, 163, 12, 73, 94, 32, 212, 55, 80, 136, 374, 71, 112, 57, 210, 40, 31, 650, 37, 627, 222, 45, 168, 50, 31, 37, 51, 88, 118.

Peptide TSQAITCQKTVPPKEKEDPLG, HPV 33, L1.
IgA: 48, 0, 0, 0, 0, 0, 0, 0, 76, 0, 35, 0, 0, 454, 0, 0, 0, 0, 61, 0, 0, 30, 0, 24, 0, 0, 32, 14, 0, 0.
IgG: 139, 128, 936, 57, 82, 82, 220, 39, 86, 127, 264, 48, 87, 34, 111, 89, 261, 50, 69, 29, 184, 316, 93, 0, 28, 21, 34, 237, 35, 0.

IgM: 5, 0, 0, 0, 55, 78, 31, 112, 209, 122, 105, 304, 137, 14, 37, 0, 1, 9, 4, 0, 99, 150, 149, 0, 0, 11, 56, 123, 190, 108.

Peptide PMDTFVVSTDSSNVTSSTPI, HPV 33, L2.
IgA: 0, 0, 0, 0, 2734, 29, 2840, 98, 266, 17, 1066, 1921, 0, 6, 0, 0, 230, 0, 0, 0, 0, 182, 2604, 0, 0, 0, 2800, 2362, 2238, 603, 0.
IgG: 740, 2531, 2626, 200, 2990, 1286, 486, 741, 1034, 248, 526, 2439, 1431, 332, 1533, 2967, 420, 727, 191, 480, 862, 2469, 375, 228, 132, 2977, 2368, 304, 2684, 790.
IgM: 43, 23, 127, 12, 52, 146, 0, 6, 184, 25, 77, 58, 53, 146, 202, 12, 19, 54, 27, 63, 172, 0, 54, 18, 52, 172, 19, 0, 134, 3.

E7 peptides for HPV types 1, 5, 6, 8, 11, 18, 31, 33 and 56

As described above, an HPV 16 peptide from the E7 open reading frame which had the sequence
CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL
was studied. Analysis of the predicted amino acid sequences revealed that all papillomaviruses have a motif of 30 amino acids in the E7 open reading frame that can be represented by CxxCxxxxxxxxxxxxxxxxxxxxLL'xxxL', where L' usually is Leucine, but in a few cases was substituted for the other hydrophobic amino acids Valine or Phenylalanine.

The peptides describ d by this formula were synthesiz d for HPV 1, 5, 8, 6, 11, 18, 31, 33 and 56 and

t sted in IgA and IgG ELISAs with a panel of 30 sera from patients with CIN.

Peptide CCGCDSNVRLVVQCTETDIREVQQLLLGTL (HPV 6, E7),
IgA: .162, 0, .182, .228, 0, 0, 0, 0, 0, 0, 0, 0, .122, .185, 0, 0, 0, 0, 0, 0, .134, .197, .227, .100, 0, .104, 0, .117, 0, .121.
IgG: .240, .287, .130, 0, 0, .185, 0, 0, .224, 0, 0, .145, 0, .130, 0, 0, 0, 0, 0, 0, 0, .401, 0, 0, .161, 0, 0, 0, 0, 0, .126.

Peptide CAYCEKLVRLTVLADHSAIRQLEELLLRSL (HPV 1, E7),
IgA: .128, 0, .142, .125, 0, 0, 0, 0, 0, 0, 0, 0,.115, .230, 0, 0, 0, 0, 0, 0, 0, .120, .121, .162, .115, 0, 0, 0, .121, 0, 0.
IgG: .229, .176, .108, 0, 0, 0, 0, 0, .123, 0, 0, 0, 0, .121, 0, 0, 0, 0, 0, 0, 0, .166, 0, 0, 0, .130, 0, 0, 0, 0, .105

Peptide CHTCNTTVRLCVNSTASDLRTIQQLLMGTV (HPV 33, E7),
IgA: 0, .178, 0, 0, 0, 0, .115, .103, .115, 0, 0, 0, .115, .339, 0, .148, .145, .105, .205, .350, .112, .130, .138, .196, 0, 0, 0, .117, 0, .132.
IgG: .148, .301, .114, 0, .134, .110, 0, .180, .161, 0, 0, .145, 0, .170, 0, 0, 0, .130, .143, .161, 0, 0, 0, .127, .112, 0, 0, 0, 0, .111.

Peptide CCGCDSNVRLVVECTDGDIRQLQDLLLGTL (HPV 11, E7),
IgA: .253, 0, .286, .142, 0, 0, 0, 0, 0, 0, 0, 0, 0, .230, 0, 0, 0, 0, 0, 0, .199, .229, .226, .158, 0, 0, 0, .107, 0, .123.
IgG: .325, .464, .259, 0, 0, .293, 0, 0, .307, .133, 0, .219, 0, .178, 0, 0, 0, 0, 0, 0, .699, 0, 0, .261, 0, 0, 0, 0, 0, .210.

Peptide CSCCQVKLRLFVNATDSGIRTFQELLFRDL (HPV 8, E7),
IgA: .103, 0, 0, .144, 0, 0, 0, 0, 0, 0, 0, 0, 0, .214, 0, 0, 0, 0, 0, 0, .133, .144, .200, .140, 0, 0, 0, 0, 0, 0.
IgG: .119, .198, 0, 0, 0, 0, 0, 0, .112, 0, 0, .135, 0, .140, 0, 0, 0, 0, 0, 0, 0, 0, 0, .108, 0, 0, 0, 0, 0, 0.

Peptide CCKCEARIELVVESSADDLRAFQQLFLNTL (HPV 18, E7),
IgA: .143, 0, .292, .133, 0, .127, 0, 0, 0, 0, 0, 0, 0, .221, 0, 0, 0, 0, 0, 0, .129, .206, .200, .141, 0, 0, .123, .135, 0, 175.
IgG: .239, .276, .200, 0, 0, .182, 0, 0, .199, 0, 0, .181, 0, 0, 0, 0, 0, 0, 0, 0, .476, .167, 0, .171, 0, 0, 0, 0, 0, 0.

Peptide CRNCEVKLRIFVHATEFGIRAFQQLLTGDL (HPV 5, E7),
IgA: 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, .128, 0, 0, 0, 0, 0, 0.
IgG: All 30 were negative.

Peptide CCECKFVVQLDIQSTKEDLRVVQQLLMGAL (HPV 56, E7),
IgA: .149, 0, .171, 0, 0, 0, 0, 0, 0, 0, 0, 0, .117, .260, 0, 0, 0, .112, 0, 0, .132, .145, .135, .120, 0, 0, 0, .132, 0, .122.
IgG: .134, .224, 0, 0, 0, .110, 0, 0, .113, 0, 0, 0, 0, .105, 0, 0, 0, 0, 0, 0, 0, .182, 0, 0, 0, 0, 0, 0, 0, 0, 0.

Peptide CCQCKSTLRLCVQSTQVDIRILQELLMGSF (HPV 31, E7),
IgA: 1.344, .200, .855, .600, 0, .358, .282, 0, .158, 0, 0, 0, 0, .694, .122, 0, 0, 0, 0, .107, .902, .948, 1.762, .882, 0, 0, 0, 0, .121, .451.
IgG: .448, .775, .435, .123, 0, .530, .411, 0, .529, .405, 0, .825, 0, .538, .179, 0, 0, 0, 0, 0, .588, .198, 0, .635, 0, 0, 0, .422, .163, .474

As shown by the results presented above, this site in the E7 protein is an important antigenic site (for both IgA and IgG) that was present for all HPV types tested except HPV 5.

Essential amino acids in the major E4 epitope.

The major E4 epitope, peptide E4:4 (SSDQDQSQTPETPATPLSCC) was investigated for necessary amino acids by the synthesis of 2 peptides that had been moved 2 amino acids compared to the original peptide, peptide E4:4N (RLSSDQDQSQTPETPATPLS) and peptide E4:4C (DQDQSQTPETPATPLSCCTE). The peptides were tested and compared with peptide E4:4 in IgA and IgG ELISAs in the same panel of 30 human sera. Both the peptide E4:4N and the peptide E4:4C were unreactive (no serum produced any optical density above 0.1) (not shown). The E4 peptide DQDQSQTPETP was synthesized in a previous work (Schoolnik, EP 0 257 754). Since the non-reactivity of peptides E4:4N and E4:4C implies that both the first residues SS and the last residues CC of peptide E4:4 are essential for its reactivity, we think that the peptide E4:4 represents a new invention with an immunoreactivity that was not present in this previously described peptide.

Epitopes in the E4 protein requiring 30 amino acids peptides.

The finding of an epitope in the E7 open reading frame that was 30 amino acids long prompted an additional test of the E4 open reading frame with overlapping 30 residues synthetic peptides. The following peptides were synthesized:

ADPAAATKYPLLKLLGSTWPTTPPRPIPKP

WPTTPPRPIPKPSPWAPKKHRRLSSDQDQS

KHRRLSSDQDQSQTPETPATPLSCCTETQW

QSQTPETPATPLSCCTETQWTVLQSSLHLT

TETQWTVLQSSLHLTAHTKDGLTVIVTLHP
The peptides were tested in IgA ELISAs with a panel of 30 human sera from CIN patients.

Peptide ADPAAATKYPLLKLLGSTWPTTPPRPIPKP,
IgA: .234, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, .106, 0, 0, 0, .171, 0, 0, 0, 0, 0, .202.

Peptide WPTTPPRPIPKPSPWAPKKHRRLSSDQDQS,
IgA: .199, 0, 0, .148, 0, 0, .662, 0, 0, .119, 0, 0, 0, .121, 0, 0, 0,0, 0, 0, 0, 0, .724, 0, 0, 0, 0, .141, .248, .136.

The peptide KHRRLSSDQDQSQTPETPATPLSCCTETQW showed an immunoreactivity that was strongly correlated to, but somewhat lesser than the reactivity of peptide SSDQDQSQTPETPATPLSCC (not shown). The peptides QSQTPETPATPLSCCTETQWTVLQSSLHLT and TETQWTVLQSSLHLTAHTKDGLTVIVTLHP reacted with 2 out of 30 sera in the IgA ELISA (not shown).

Antibodies in cervical secretions

The major immunoreactive peptides were also tested in IgA and IgG ELISAs with cervical secretions from 30 women with cervical intraepithelial neoplasia (CIN) or with a history of CIN. It was found that those peptides which were the most immunoreactive with serum also were those which were most reactive with cervical secretions.

The results are exemplified with peptide E2:9 (FDGDICNTMHYTNWTHIYIC). IgA-ELISA where the results are given as optical densities for a 1:2 dilution of the secr tion:

.063, 0, .059, 0, .091, .456, .074, 0, 0, 0, .177, 0, 1.563, 0,.124, 0, 0, 0, 0, 0, .104, .083, 0, 0, 0, .068, 0, 0, 0, .150.

IgG-ELISA also at a 1:2 dilution of the secretion:

.080, 0, 0, 0, .067, .262, .110, 0, 0, 0, .131, 0, .092, 0, 0, 0,0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, 0, .070.

As seen from the results shown above, IgA was the major Ig class in secretions. An ELISA was also performed on serum from the same patients. Comparison of the IgG-results for serum and secretions showed a strong correlation, whereas no obvious correlation was seen between IgA in serum and secretions (not shown). This suggests that most of the IgA found in secretions was produced locally, whereas the IgG found in secretions may be due to a leakage of serum antibodies into the secretions.

In summary, these results show that several immunoreactive peptides based on both HPV 1, 5, 6, 8, 11, 16, 18, 31, 33 and 56 have been found. The immunoreactivity is quite different depending on if IgG, IgA or IgM is measured. The method used here, ELISA, is only one of several variants of immunoasssay, but the most simple and most practical method for testing sera on a large scale.

CLAIMS

1. Method for diagnosing the presence of human papillomavirus (HPV) infection and of papillomavirus (PV) carrying tumours by immuno assay, c h a r a c t e r i z e d in that the detection is performed by immunoassay based on the E2, L1 or L2 protein of HPV 1, the E2 protein of HPV 5, the E2 protein of HPV 8, the E4 or L2 protein of HPV 6, the E4, L1 or L2 protein of HPV 11, the E7 protein från HPV 1, 6, 8, 11, 18, 31, 33 or 56, the E7 protein från HPV 1, 6, 8, 11, 18, 31, 33 or 56, the E4, E7, L1 or L2 protein of HPV 18, the E2, L1 or L2 protein of HPV 31, the E2, E4, L1 or L2 protein of HPV 33 or on any of the peptides

IGSARMLVKFIDEAQREKC (HPV 1, E2)

YDNNPDNQTRHTIWNHVYYQ (HPV 1, E2)

CAYCEKLVRLTVLADHSAIRQLEELLLRSL (HPV 1, E7)

LGSSLAAKCPEQAPPEPQTDPY (HPV 1, L1)

DIPLVELNLGLETDTSSVVQ (HPV 1, L2)

LGRPRMLISFSSYTQRRDC (HPV 5, E2)

FDGCANNTMDYVVWTDVYVQ (HPV 6, E2)

RLGNEHEESNSPLATPCVWP (HPV 6, E4)

CCGCDSNVRLVVQCTETDIREVQQLLLGTL (HPV 6, E7)

PLDTFVVSSSDSGPTSSTPV (HPV 6, L2)

LGRSRMLILFTSAGQRKDC (HPV 8, E2)

CSCCQVKLRLFVNATDSGIRTFQELLFRDL (HPV 8, E7)

FDGCEDNVMEYVVWTHIYLQ (HPV 11, E2)

RRRLGSEHVDRPLTTPCVWP (HPV 11, E4)

CCGCDSNVRLVVECTDGDIRQLQDLLLGTL (HPV 11, E7)

QSQAITCQKPTPEKEKQDPYK (HPV 11, L1)

PLDTFVVSSSDSGPTSSTPL (HPV 11, L2)

KQHRDAVQVLKRKYLGSCIE (HPV 16, E1)

QYSGGSGGGCSQYSSGSGGE (HPV 16, E1)

GSGGEGVSERHTICQTPLTN (HPV 16, E1)

KAAMLAKFKELYGVSFSELV (HPV 16, E1)

DSIKTLLQQYCIYLHIQSLA (HPV 16, E1)

SKSHFWLQPLADAKIGMLDD (HPV 16, E1)

GMLDDATVPCWNYIDDNLRN (HPV 16, E1)
LHNRLVVFTFPNEFPFDENG (HPV 16, E1)
FDENGNPVYELNDKNWKS (HPV 16, E1)
DSLPTFKCVSGQNTNTL (HPV 16, E1)
GDICNTMHYTNWTHIYICEE (HPV 16, E2)
LTHYENDSTDLRDHIDYWKH (HPV 16, E2)
PTGCIKKHGYTVEVQFDGDI (HPV 16, E2)
FDGDICNTMHYTNWTHIYIC (HPV 16, E2)
HIYICEEASVTVVEGQVDYY (HPV 16, E2)
QVDYYGLYYVHEGIRTYFVQ (HPV 16, E2)
TYFVQFKDDAEKYSKNKVWE (HPV 16, E2)
NKVWEVHAGGQVILCPTSVF (HPV 16, E2)
AVALGTEETQTTIQRPRSEP (HPV 16, E2)
PRSEPDTGNPCHTTKLLHRD (HPV 16, E2)
FNSSHKGRINCNSNTTPIVH (HPV 16, E2)
SSDQDQSQTPETPATPLSCC (HPV 16, E4)
IPKPSPWAPKKHRRLSSDQD (HPV 16, E4)
ADPAAATKYPLLKLLGSTWPTTPPRPIPKP (HPV 16, E4)
WPTTPPRPIPKPSPWAPKKHRRLSSDQDQS (HPV 16, E4)
TNLDTASTTLLACFLLCFCV (HPV 16, E5)
YSKISEYRHYCYSLYGTTLE (HPV 16, E6)
GTTLEQQYNKPLCDLLIRCI (HPV 16, E6)
HGDTPTLHEYMLDLQPETTD (HPV 16, E7)
PETTDLYCYEQLNDSSEEED (HPV 16, E7)
PDRAHYNIVTFCCKCDSTLR (HPV 16, E7)
DSTLRLCVQSTHVDIRTLEDL (HPV 16, E7)
TLEDLLMGTLGIVCPICSQKP (HPV 16, E7)
HGDTPTLHEYMLDLQPETTDLYCYEQLNDS (HPV 16, E7)
QAEPDRAHYNIVTFCCKCDSTLRLCVQSTH (HPV 16, E)7
CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL (HPV 16, E7)
VQSTHVDIRTLEDLLMGTLGIVCPICSQKP (HPV 16, E7)
VTSQAIACQKHTPPAPKEDPL (HPV 16, L1)
VHTGFGAMDFTTLQAGGC (HPV 16, L1)
GGTLEDTYRFGGC (HPV 16, L1)
FDGNKDNCMTYVAWDSVYYM (HPV 18, E2)

SLLNSYSTPPHRIPAPCPWA (HPV 18, E4)
CCKCEARIELVVESSADDLRAFQQLFLNTL (HPV 18, E7)
HGPKATLQDIVLHLEPQNEIPVDLLCHEQL (HPV 18, E7)
PQNEIPVDLLCHEQLSDSEEENDEIDGVNH (HPV 18, E7)
SDSEEENDEIDGVNHQHLPARRAEPQRHTM (HPV 18, E7)
QHLPARRAEPQRHTMLCMCCKCEARIELVV (HPV 18, E7)
LCMCCKCEARIELVVESSADDLRAFQQLFL (HPV 18, E7)
ESSADDLRAFQQLFLNTLSFVCPWCASQQ (HPV 18, E7)
QSVAITCQKDAAPAENKDPYD (HPV 18, L1)
PLQTFASSGTGEEPISSTPL (HPV 18, L2)
FDGDVHNTMHYTNWKFIYLC (HPV 31, E2)
HKNAIVTLTYISTSQRDDC (HPV 31, E2)
CCQCKSTLRLCVQSTQVDIRILQELLMGSF (HPV 31, E7)
TSQAITCQKTAPQKPKEDPFK (HPV 31, L1)
PMDTFIVSTNNQNITSSTPI (HPV 31, L2)
YDNDKKNTMDYTNWGEIYII (HPV 33, E2)
PQTPPSPLQSCSVQTPPWTI (HPV 33, E4)
CHTCNTTVRLCVNSTASDLRTIQQLLMGTV (HPV 33, E7)
TSQAITCQKTVPPKEKEDPLG (HPV 33, L1)
PMDTFVVSTDSSNVTSSTPI (HPV 33, L2)
CCECKFVVQLDIQSTKEDLRVVQQLLMGAL (HPV 56, E7)

or on modifications of any of the above-mentioned polypeptides where the modified peptide contains an epitope that is essentially similar to the epitope of the original polypeptide or on modifications of the above-mentioned peptides, which are reactive with antibodies against the original peptide.

2. Method as in claim 1, wherein the immunoassay is based on IgA-antibodies against the proteins from E2, L1 or L2 of HPV 1, the E2 protein of HPV 8, the E4, L1 or L2 protein of HPV 6, the E4, L1 or L2 protein of HPV 11, the E1, E4, E5, E6 or E7 protein of HPV 16, the E7, L1 or L2 protein of HPV 18, the E2, L1 or L2 protein of HPV 31, the E2, E4, L1 or L2 protein of HPV 33 or against any of the p ptid s

CAYCEKLVRLTVLADHSAIRQLEELLLRSL (HPV 1, E7)
FDGCANNTMDYVVWTDVYVQ (HPV 6, E2)
CCGCDSNVRLVVQCTETDIREVQQLLLGTL (HPV 6, E7)
CSCCQVKLRLFVNATDSGIRTFQELLFRDL (HPV 8, E7)
FDGCEDNVMEYVVWTHIYLQ (HPV 11, E2)
CCGCDSNVRLVVECTDGDIRQLQDLLLGTL (HPV 11, E7)
QYSGGSGGGCSQYSSGSGGE (HPV 16, E1)
GSGGEGVSERHTICQTPLTN (HPV 16, E1)
KAAMLAKFKELYGVSFSELV (HPV 16, E1)
DSIKTLLQQYCLYLHIQSLA (HPV 16, E1)
SKSHFWLQPLADAKIGMLDD (HPV 16, E1)
GMLDDATVPCWNYIDDNLRN (HPV 16, E1)
LHNRLVVFTFPNEFPFDENG (HPV 16, E1)
FDENGNPVYELNDKNWKS (HPV 16, E1)
DSLPTFKCVSGQNTNTL (HPV 16, E1)
GDICNTMHYTNWTHIYICEE (HPV 16, E2)
LTHYENDSTDLRDHIDYWKH (HPV 16, E2)
FDGDICNTMHYTNWTHIYIC (HPV 16, E2)
HIYICEEASVTVVEGQVDYY (HPV 16, E2)
QVDYYGLYYVHEGIRTYFVQ (HPV 16, E2)
TYFVQFKDDAEKYSKNKVWE (HPV 16, E2)
NKVWEVHAGGQVILCPTSVF (HPV 16, E2)
AVALGTEETQTTIQRPRSEP (HPV 16, E2)
PRSEPDTGNPCHTTKLLHRD (HPV 16, E2)
FNSSHKGRINCNSNTTPIVH (HPV 16, E2)
SSDQDQSQTPETPATPLSCC (HPV 16, E4)
ADPAAATKYPLLKLLGSTWPTTPPRPIPKP (HPV 16, E4)
WPTTPPRPIPKPSPWAPKKHRRLSSDQDQS (HPV 16, E4),
TNLDTASTTLLACFLLCFCV (HPV 16, E5)
YSKISEYRHYCYSLYGTTLE (HPV 16, E6)
HGDTPTLHEYMLDLQPETTD (HPV 16, E7)
PDRAHYNIVTFCCKCDSTLR (HPV 16, E7)
DSTLRLCVQSTHVDIRTLEDL (HPV 16, E7)
TLEDLLMGTLGIVCPICSQKP (HPV 16, E7)
HGDTPTLHEYMLDLQPETTDLYCYEQLNDS (HPV 16, E7)

QAEPDRAHYNIVTFCCKCDSTLRLCVQSTH (HPV 16, E7)
CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL (HPV 16, E7)
VQSTHVDIRTLEDLLMGTLGIVCPICSQKP (HPV 16, E7)
VTSQAIACQKHTPPAPKEDPL (HPV 16, L1)
FDGNKDNCMTYVAWDSVYYM (HPV 18, E2)
CCKCEARIELVVESSADDLRAFQQLFLNTL (HPV 18, E7)
CCQCKSTLRLCVQSTQVDIRILQELLMGSF (HPV 31, E7)
CHTCNTTVRLCVNSTASDLRTIQQLLMGTV (HPV 33, E7)
CCECKFVVQLDIQSTKEDLRVVQQLLMGAL (HPV 56, E7)

or on modifications of any of the above-mentioned polypeptides, which contains an epitope that is essentially similar to the epitope of the original polypeptide or on modifications of the above-mentioned peptides, which are reactive with antibodies against the original peptide.

3. Method as in claim 1, wherein the immunoassay is based on IgG-antibodies against any of the peptides

IGSARMLVKFIDEAQREKC (HPV1, E2)
YDNNPDNQTRHTIWNHVYYQ (HPV 1, E2)
CAYCEKLVRLTVLADHSAIRQLEELLLRSL (HPV 1, E7)
LGSSLAAKCPEQAPPEPQTDPY (HPV 1, L1)
DIPLVELNLGLETDTSSVVQ (HPV 1, L2)
LGRPRMLISFSSYTQRRDC (HPV 5, E2)
LGRSRMLILFTSAGQRKDC (HPV 8, E2)
FDGCANNTMDYVVWTDVYVQ (HPV 6, E2)
RLGNEHREESNSPLATPCVWP (HPV 6, E4)
CCGCDSNVRLVVQCTETDIREVQQLLLGTL (HPV 6, E7)
PLDTFVVSSSDSGPTSSTPV (HPV 6, L2)
CSCCQVKLRLFVNATDSGIRTFQELLFRDL (HPV 8, E7)
FDGCEDNVMEYVVWTHIYLQ (HPV 11, E2)
CCGCDSNVRLVVECTDGDIRQLQDLLLGTL (HPV 11, E7)
QSQAITCQKPTPEKEKQDPYK (HPV 11, L1)
PLDTFVVSSSDSGPTSSTPL (HPV 11, L2)
GMLDDATVPCWNYIDDNLRN (HPV 16, E1)
FDENGNPVYELNDKNWKS (HPV 16, E1)
GDICNTMHYTNWTHIYICEE (HPV 16, E2)

FDGDICNTMHYTNWTHIYIC (HPV 16, E2)
NKVWEVHAGGQVILCPTSVFHPV (HPV 16, E2)
AVALGTEETQTTIQRPRSEP (HPV 16, E2)
PRSEPDTGNPCHTTKLLHRD (HPV 16, E2)
SSDQDQSQTPETPATPLSCC (HPV 16, E4)
TNLDTASTTLLACFLLCFCV (HPV 16, E5)
HGDTPTLHEYMLDLQPETTD (HPV 16, E7)
PETTDLYCYEQLNDSSEEED (HPV 16, E7)
DSTLRLCVQSTHVDIRTLEDL (HPV 16, E7)
HGDTPTLHEYMLDLQPETTDLYCYEQLNDS (HPV 16, E7)
QAEPDRAHYNIVTFCCKCDSTLRLCVQSTH (HPV 16, E7)
CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL (HPV 16, E7)
"VTSQAIACQKHTPPAPKEDPL (HPV 16, L1)
FDGNKDNCMTYVAWDSVYYM (HPV 18, E2)
CCKCEARIELVVESSADDLRAFQQLFLNTL (HPV 18, E7)
QHLPARRAEPQRHTMLCMCCKCEARIELVV (HPV 18, E7)
LCMCCKCEARIELVVESSADDLRAFQQLFL (HPV 18, E7)
ESSADDLRAFQQLFLNTLSFVCPWCASQQ (HPV 18, E7)
QSVAITCQKDAAPAENKDPYD (HPV 18, L1)
PLQTFASSGTGEEPISSTPL (HPV 18, L2)
HKNAIVTLTYISTSQRDDC (HPV 31, E2)
CCQCKSTLRLCVQSTQVDIRILQELLMGSF (HPV 31, E7)
PMDTFIVSTNNQNITSSTPI (HPV 31, L2)
YDNDKKNTMDYTNWGEIYII (HPV 33, E2)
CHTCNTTVRLCVNSTASDLRTIQQLLMGTV (HPV 33, E7)
TSQAITCQKTVPPKEKEDPLG (HPV 33, L1)
PMDTFVVSTDSSNVTSSTPI (HPV 33, L2)
CCECKFVVQLDIQSTKEDLRVVQQLLMGAL (HPV 56, E7)

or on modifications of any of the above-mentioned polypeptides, which contains an epitope that is essentially similar to the epitope of the original polypeptide or on modifications of the above-mentioned peptides, which are reactive with antibodies against the original peptide.

4. Meth d as in claim 1, wherein the immunoassay is based on IgM-antibodies against any of the peptides

IGSARMLVKFIDEAQREKC (HPV1, E2)
YDNNPDNQTRHTIWNHVYYQ (HPV 1, E2)
LGSSLAAKCPEQAPPEPQTDPY (HPV 1, L1)
LGRPRMLISFSSYTQRRDC (HPV 5, E2)
LGRSRMLILFTSAGQRKDC (HPV 8, E2)
RLGNEHEESNSPLATPCVWP (HPV 6, E4)
PLDTFVVSSSDSGPTSSTPV (HPV 6, L2)
FDGCEDNVMEYVVWTHIYLQ (HPV 11, E2)
RRRLGSEHVDRPLTTPCVWP (HPV 11, E4)
QSQAITCQKPTPEKEKQDPYK (HPV 11, L1)
PLDTFVVSSSDSGPTSSTPL (HPV 11, L2)
KQHRDAVQVLKRKYLGSCIE (HPV 16, E1)
QYSGGSGGGCSQYSSGSGGE (HPV 16, E1)
SKSHFWLQPLADAKIGMLDD (HPV 16, E1)
DSLPTFKCVSGQNTNTL (HPV 16, E1)
PTGCIKKHGYTVEVQFDGDI (HPV 16, E2)
TYFVQFKDDAEKYSKNKVWEHPV (HPV 16, E2)
PRSEPDTGNPCHTTKLLHRD (HPV 16, E2)
FNSSHKGRINCNSNTTPIVH (HPV 16, E2)
IPKPSPWAPKKHRRLSSDQD (HPV 16, E4)
TNLDTASTTLLACFLLCFCV (HPV 16, E5)
YSKISEYRHYCYSLYGTTLE (HPV 16, E6)
GTTLEQQYNKPLCDLLIRCI (HPV 16, E6)
PDRAHYNIVTFCCKCDSTLR (HPV 16, E7)
GDTPTLHEYMLDLQPETTDLYCYEQLNDS (HPV 16, E7)
QAEPDRAHYNIVTFCCKCDSTLRLCVQSTH (HPV 16, E7)
CCKCDSTLRLCVQSTHVDIRTLEDLLMGTL (HPV 16, E7)
VQSTHVDIRTLEDLLMGTLGIVCPICSQKP (HPV 16, E7)
FDGNKDNCMTYVAWDSVYYM (HPV 18, E2)
PQNEIPVDLLCHEQLSDSEEENDEIDGVNH (HPV 18, E7)
QHLPARRAEPQRHTMLCMCCKCEARIELVV (HPV 18, E7)
LCMCCKCEARIELVVESSADDLRAFQQLFL (HPV 18, E7)
ESSADDLRAFQQLFLNTLSFVCPWCASQQ (HPV 18, E7)
QSVAITCQKDAAPAENKDPYD (HPV 18, L1)
PLQTFASSGTGEEPISSTPL (HPV 18, L2)

HKNAIVTLTYISTSQRDDC (HPV 31, E2)
TSQAITCQKTAPQKPKEDPFK (HPV 31, L1)
PMDTFIVSTNNQNITSSTPI (HPV 31, L2)
YDNDKKNTMDYTNWGEIYII (HPV 33, E2)
PQTPPSPLQSCSVQTPPWTI (HPV 33, E4)
TSQAITCQKTVPPKEKEDPLG (HPV 33, L1)
PMDTFVVSTDSSNVTSSTPI (HPV 33, L2)

or on modifications of any of the above-mentioned polypeptides, which contains an epitope that is essentially similar to the epitope of the original polypeptide or on modifications of the above-mentioned peptides, which are reactive with antibodies against the original peptide.

5. Method as in any of claims 1-4, wherein the immuoassay is ELISA.

6. Method as in any of claims 1-4, wherein the immunoassay is performed on serum.

7. Method as in any of claims 1-4, wherein the immunoassay is performed on cervical secretions.

8. Method as in any of claims 1-4, wherein the immuoassay measures the presence of cervix cancer, condyloma, CIN, warts or squamous cell carcinoma in the skin.

9. Method as in any of claims 1-4, wherein the immuoassay is immunofluorescence.

10. Method as in any of claims 1-4, wherein the immuoassay is immunohistocytochemistry.

11. Method as in any of claims 1-4, wherein the immunoassay is performed on secretions gathered with a brush or a spatula.

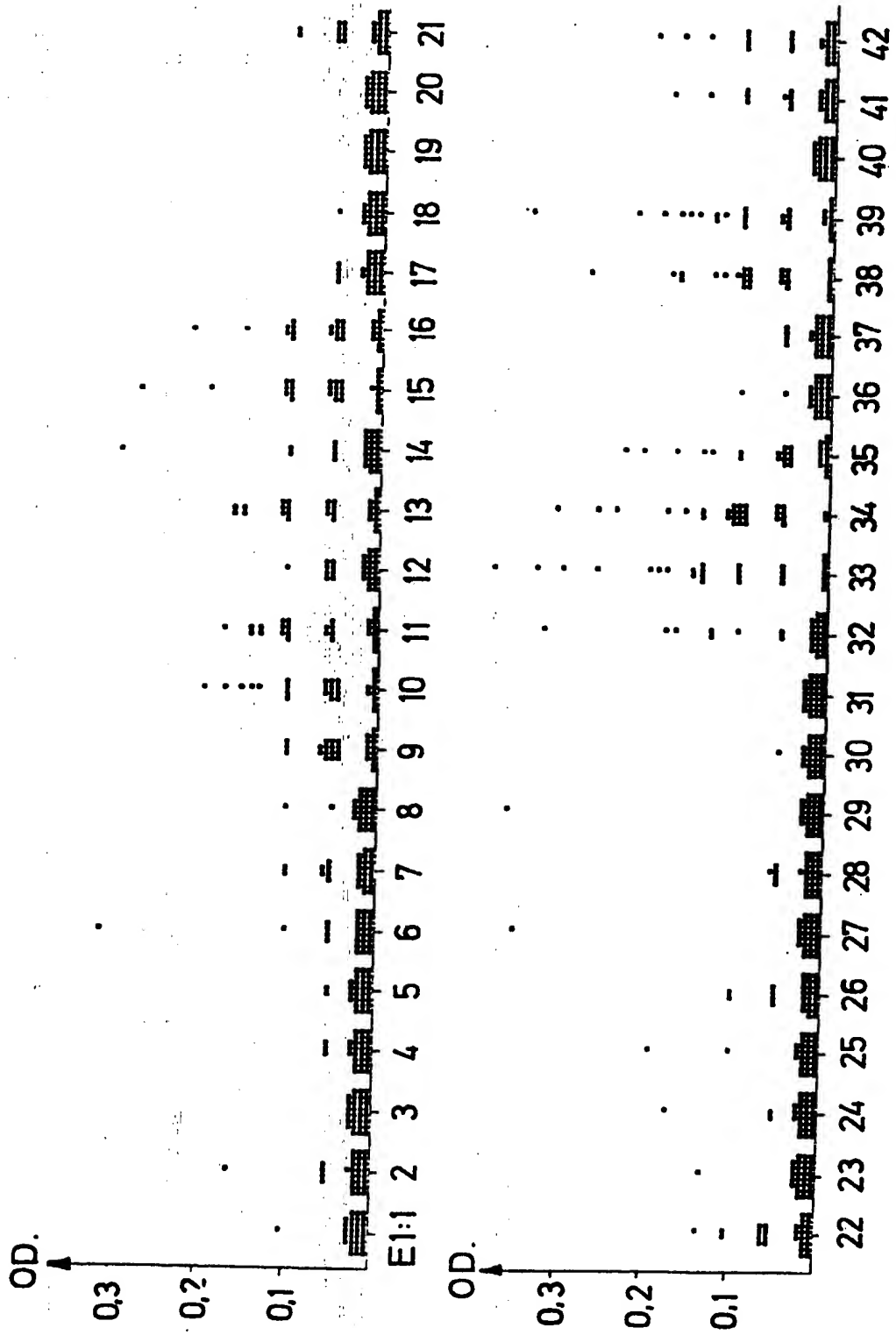

OD.
0,3
0,2
0,1
E1:1
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
OD.
0,3
0,2
0,1
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
E 1, IgA

FIG. 1

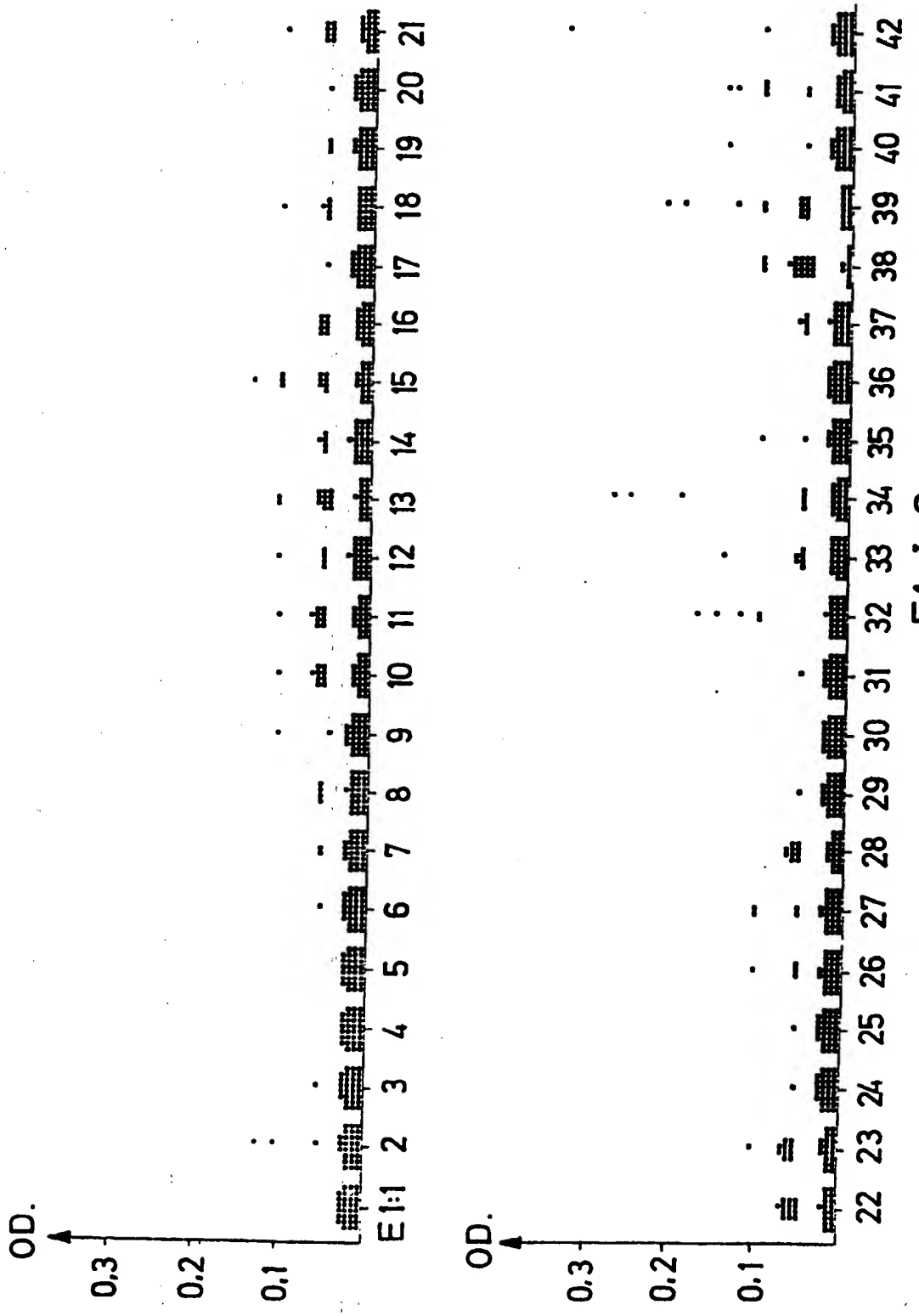
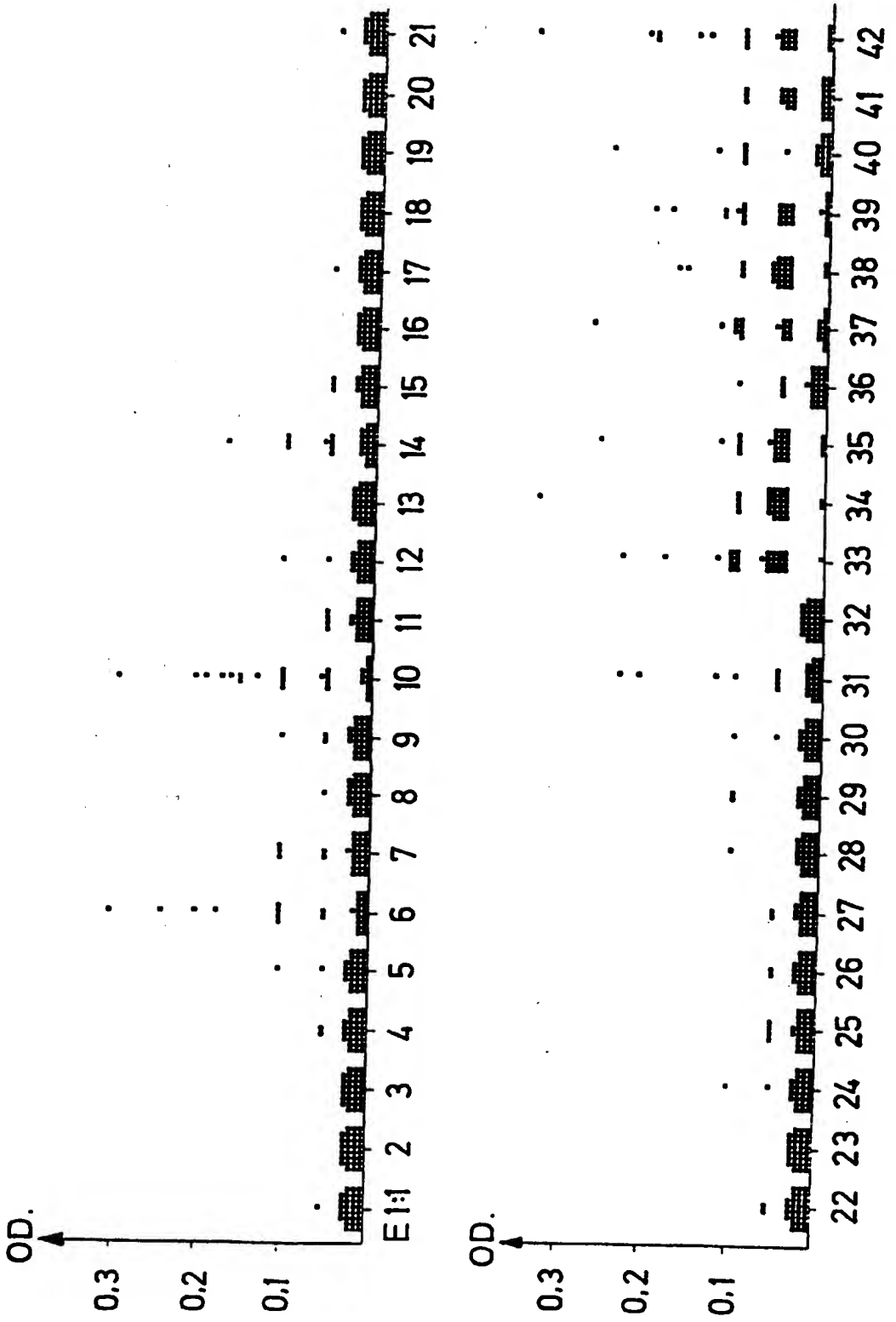

OD.
0,3
0,2
0,1
E 1:1
2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21
OD.
0,3
0,2
0,1
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42
E1, IgG

FIG. 2


OD.
0,3
0,2
0,1
E1:1
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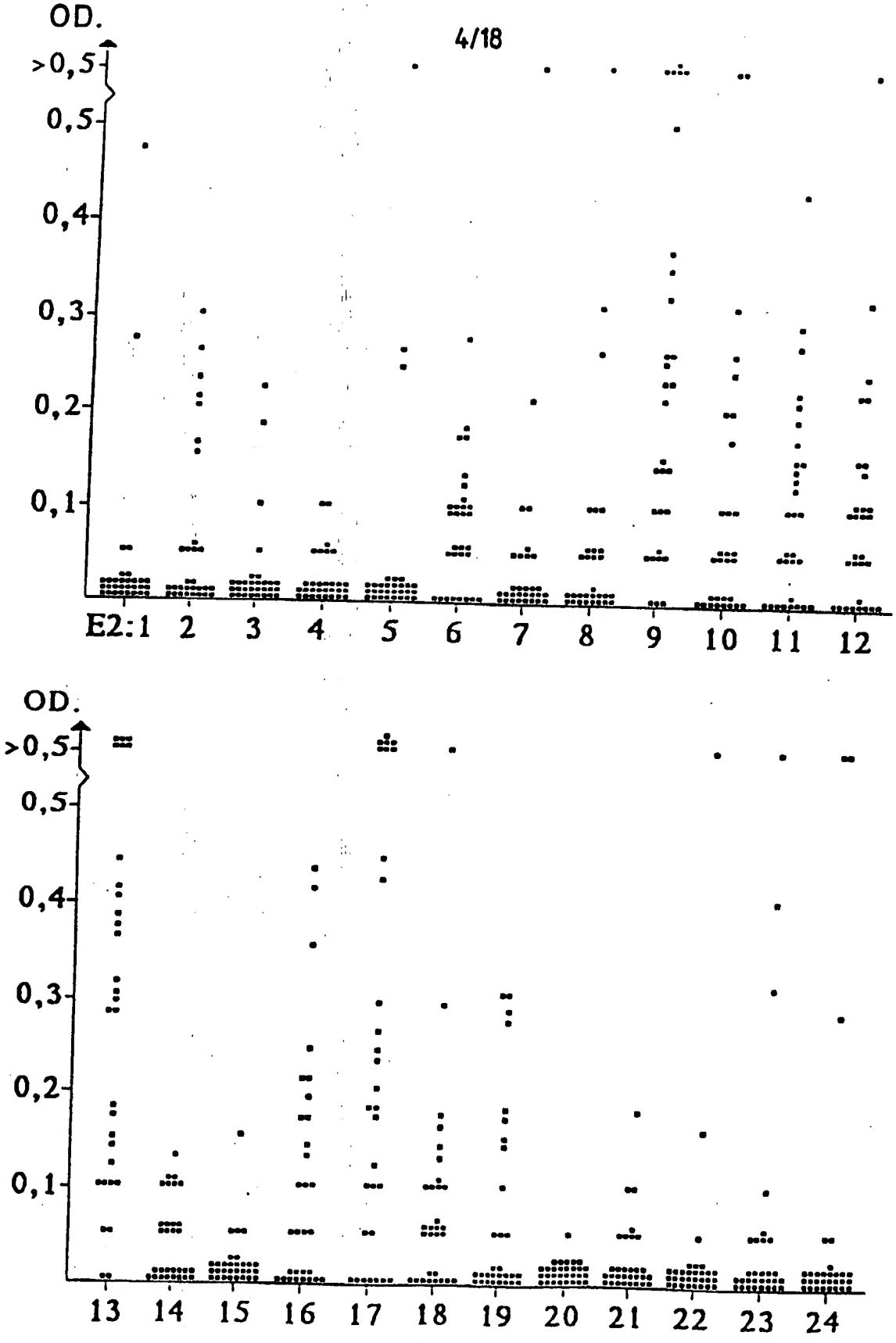
22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42

E1. IgM

FIG. 3


OD.
>0,5
0,5
0,4
0,3
0,2
0,1
E2:1 2 3 4 5 6 7 8 9 10 11 12
OD.
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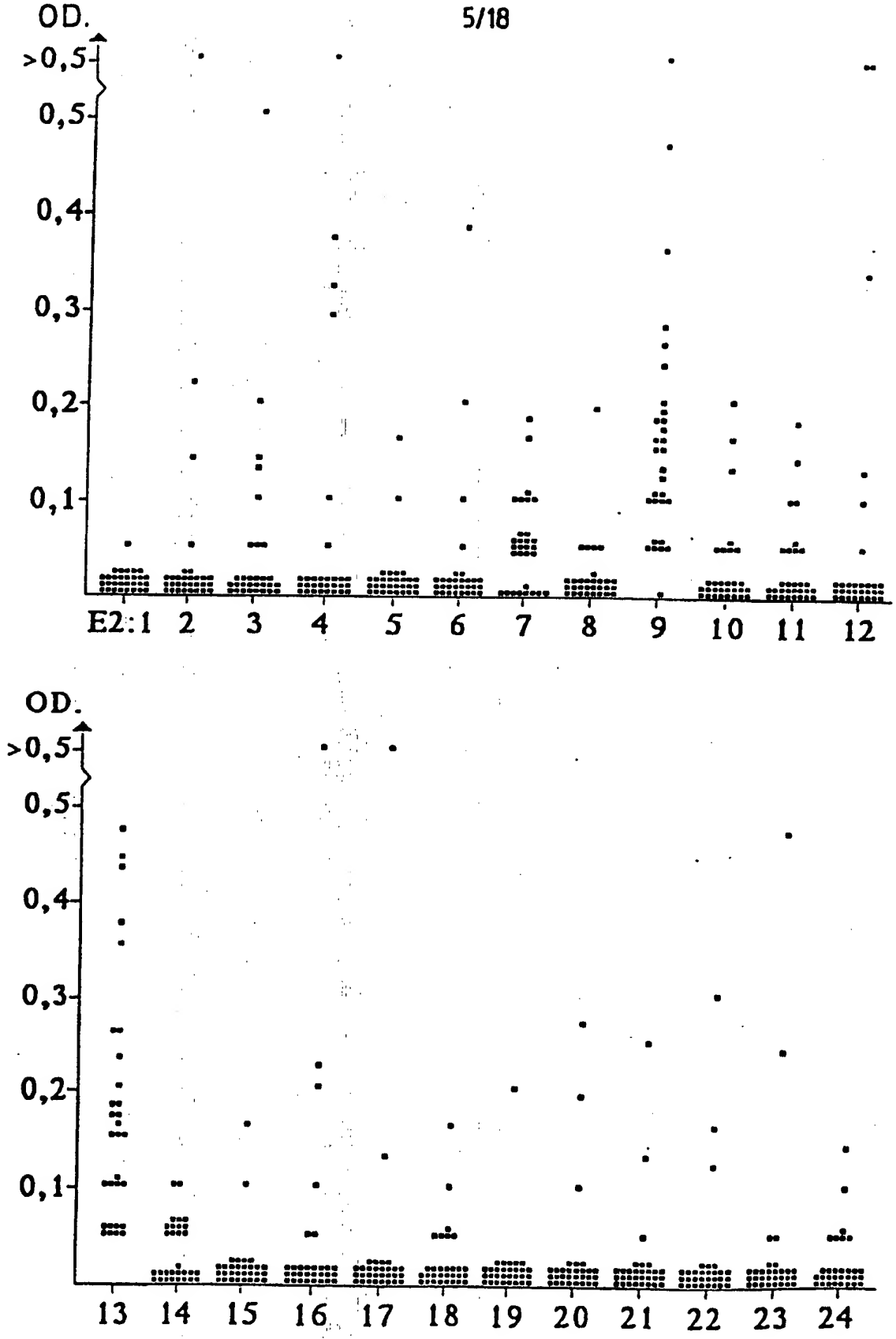
0,5
0,4
0,3
0,2
0,1
13 14 15 16 17 18 19 20 21 22 23 24

FIG. 4


OD.
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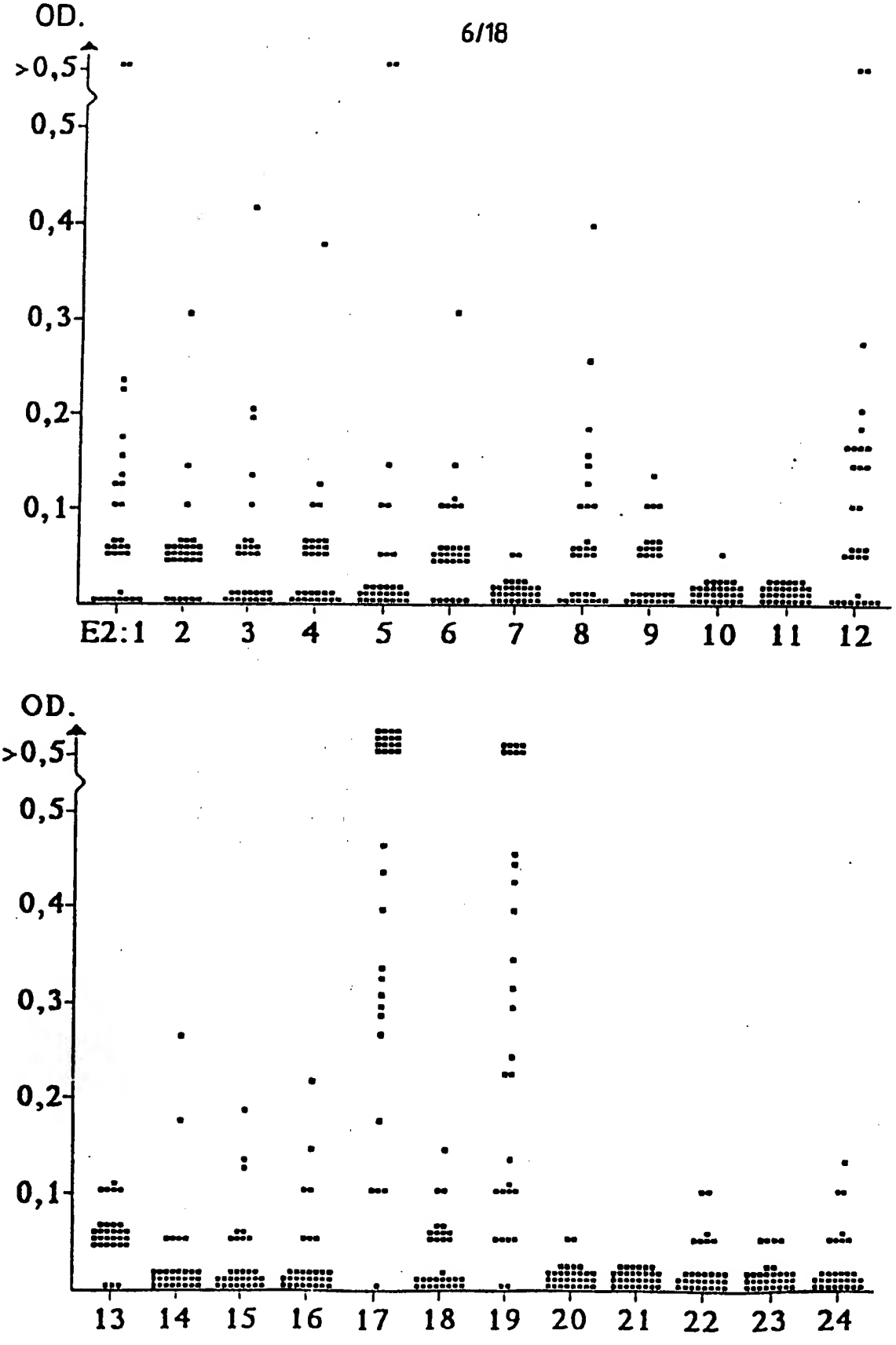
0,4
0,3
0,2
0,1
E2:1 2 3 4 5 6 7 8 9 10 11 12
OD.
>0,5
0,5
0,4
0,3
0,2
0,1
13 14 15 16 17 18 19 20 21 22 23 24

FIG. 5


OD.
>0,5
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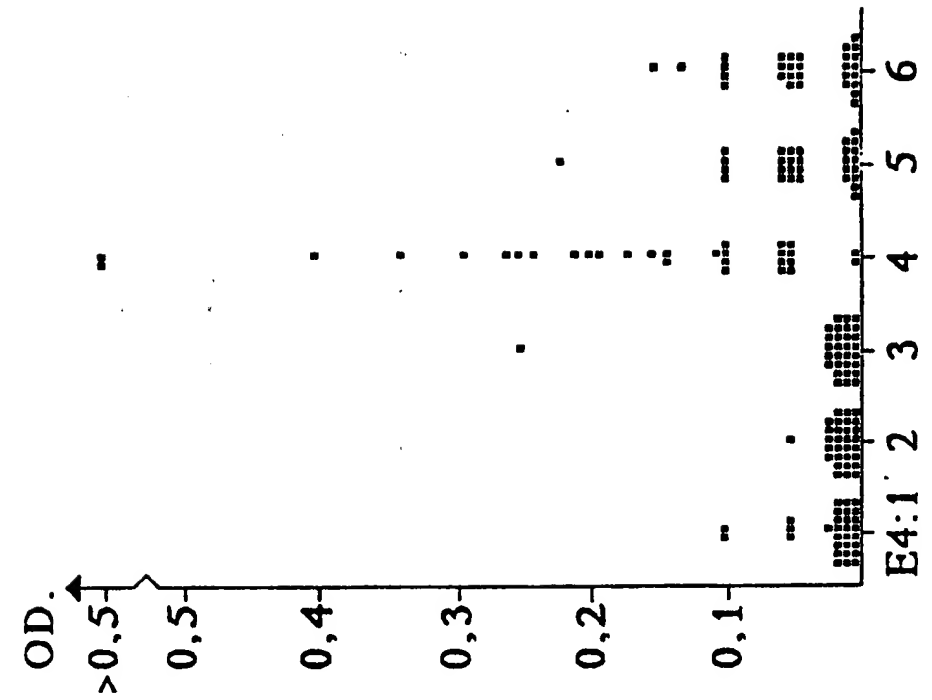
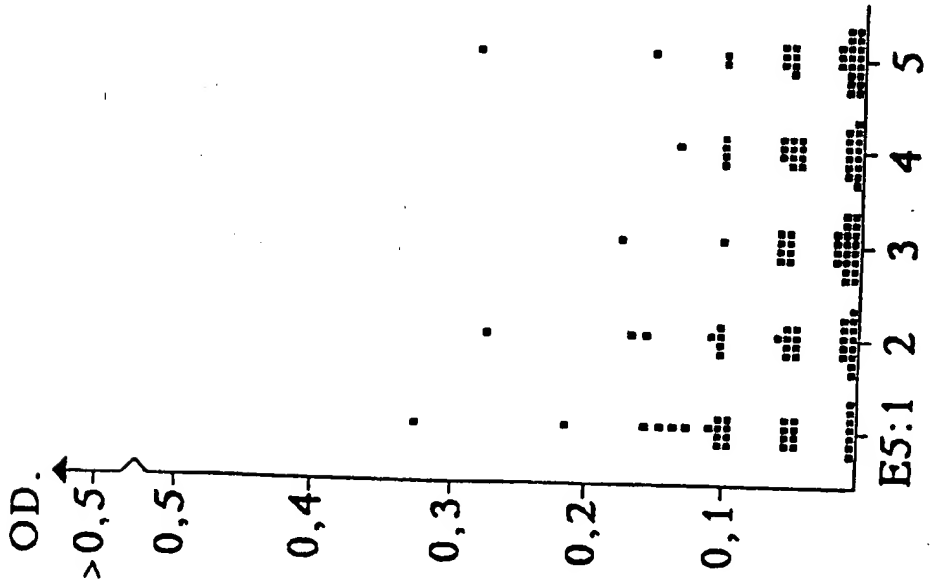
0,4
0,3
0,2
0,1
E2:1 2 3 4 5 6 7 8 9 10 11 12
OD.
>0,5
0,5
0,4
0,3
0,2
0,1
13 14 15 16 17 18 19 20 21 22 23 24

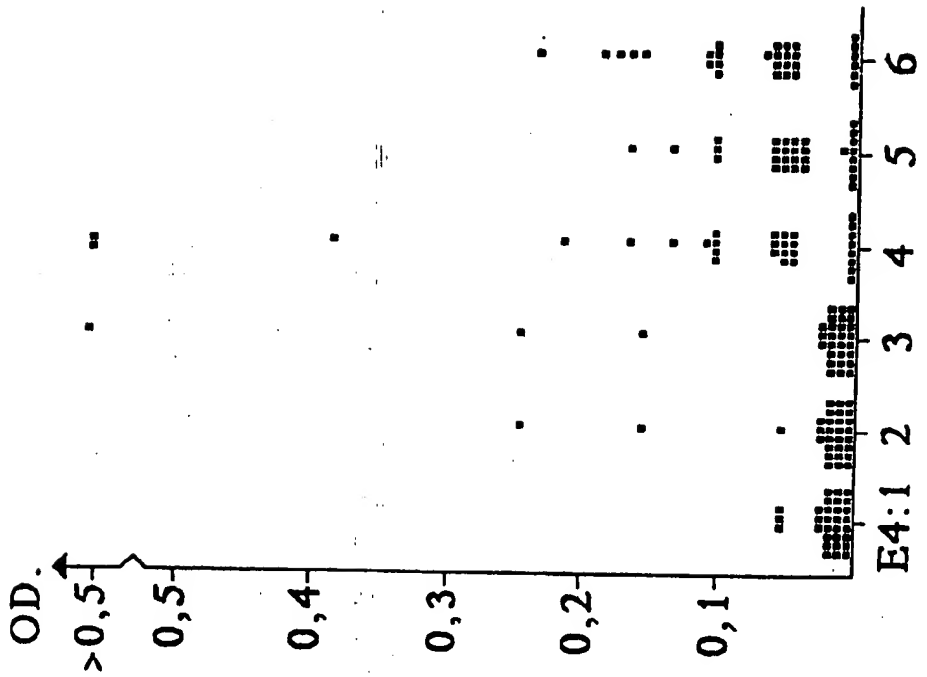
FIG. 6



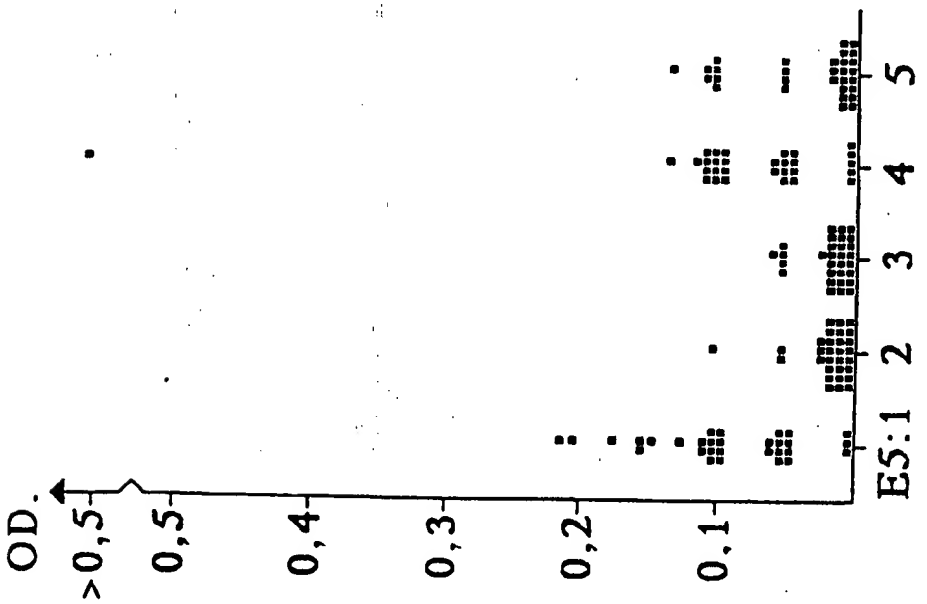
E4, IgA
FIG. 7



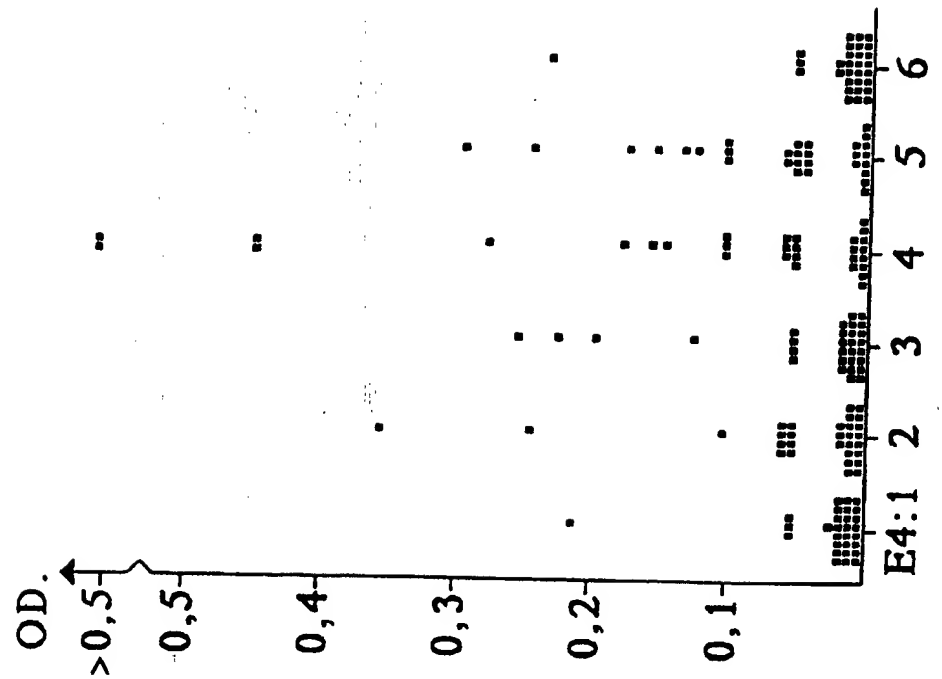
E5, IgA
FIG. 10



E4, IgG
FIG. 8

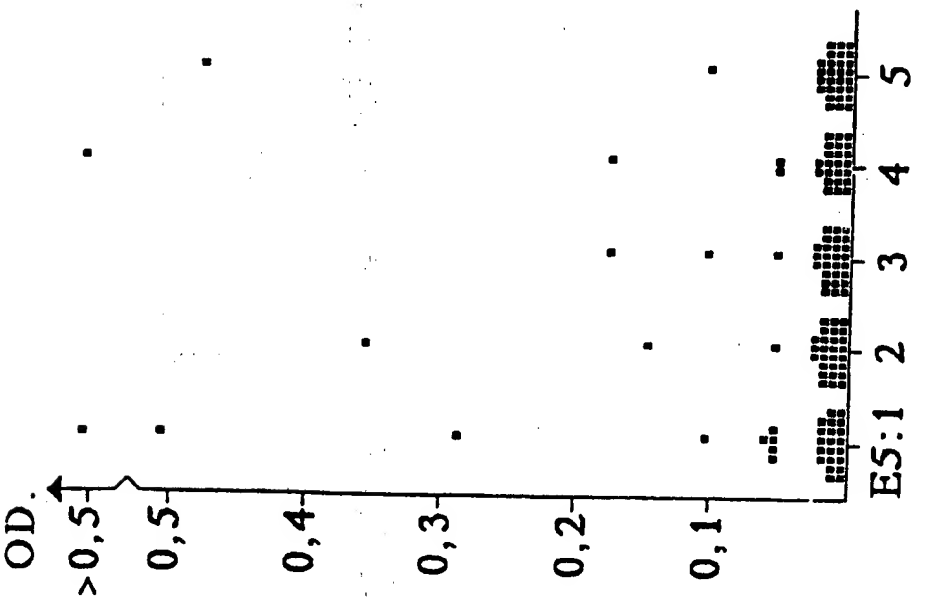


E5, IgG
FIG. 11



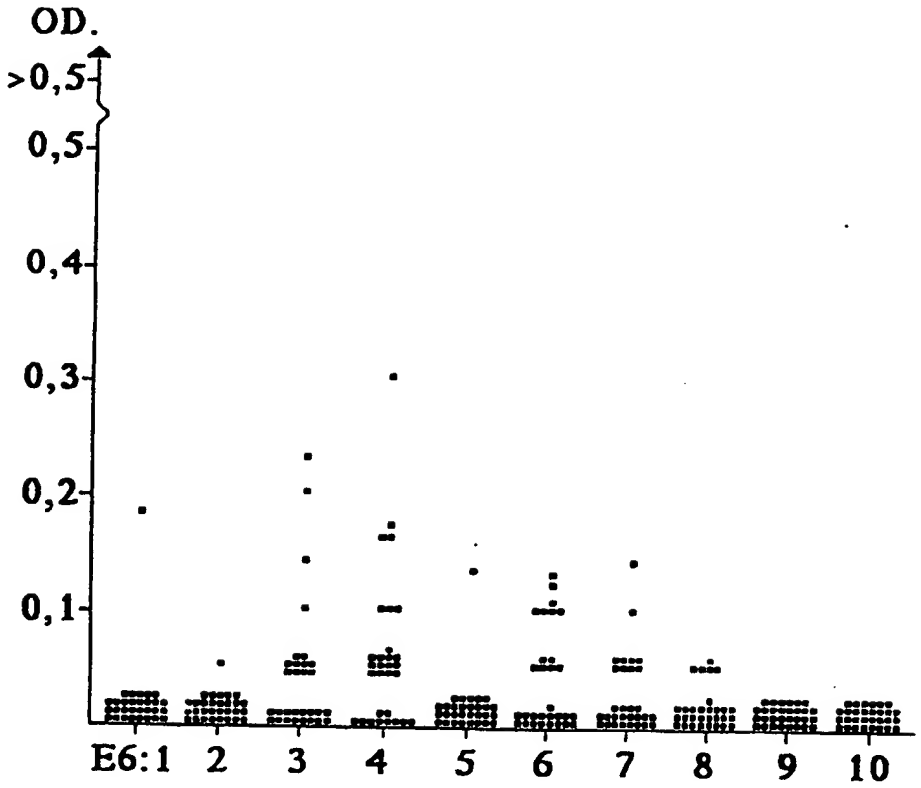
E4, IgM

FIG. 9



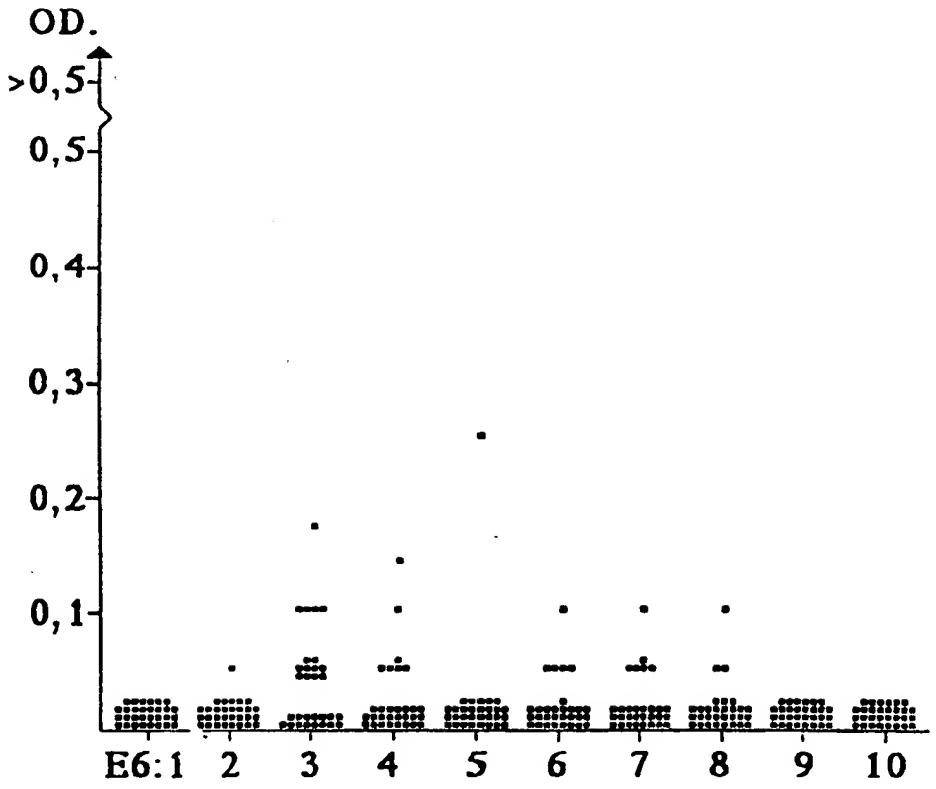
E5, IgM

FIG. 12



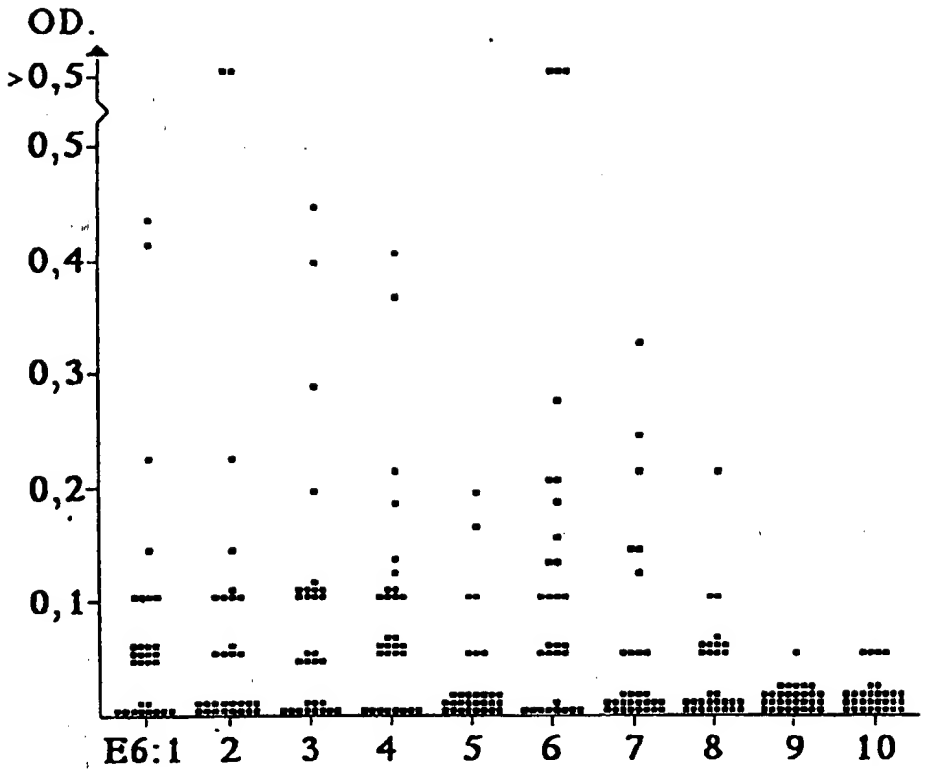
E6, IgA

FIG. 13



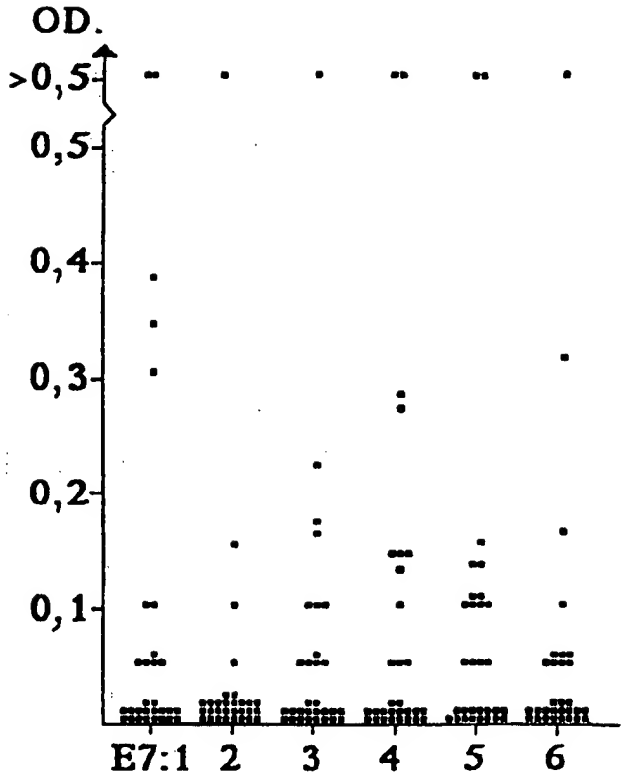
E6, IgG

FIG. 14



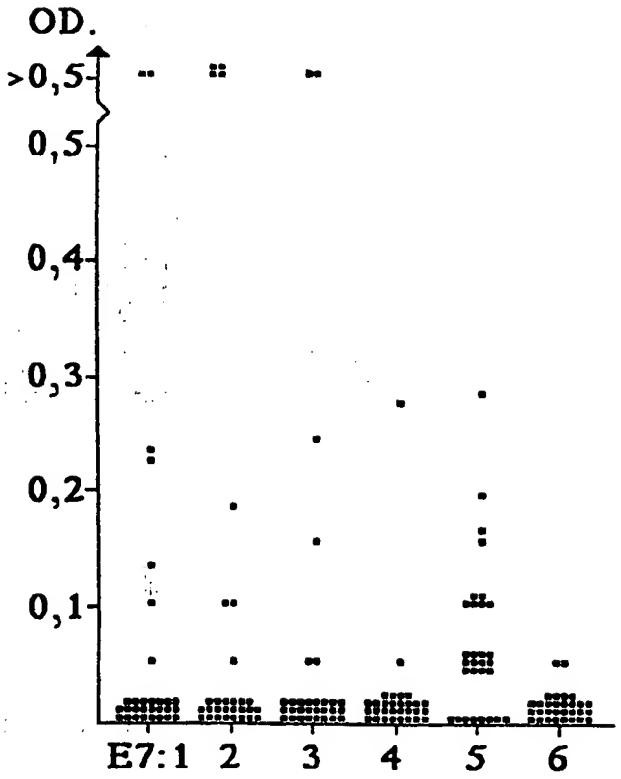
E6, IgM

FIG. 15



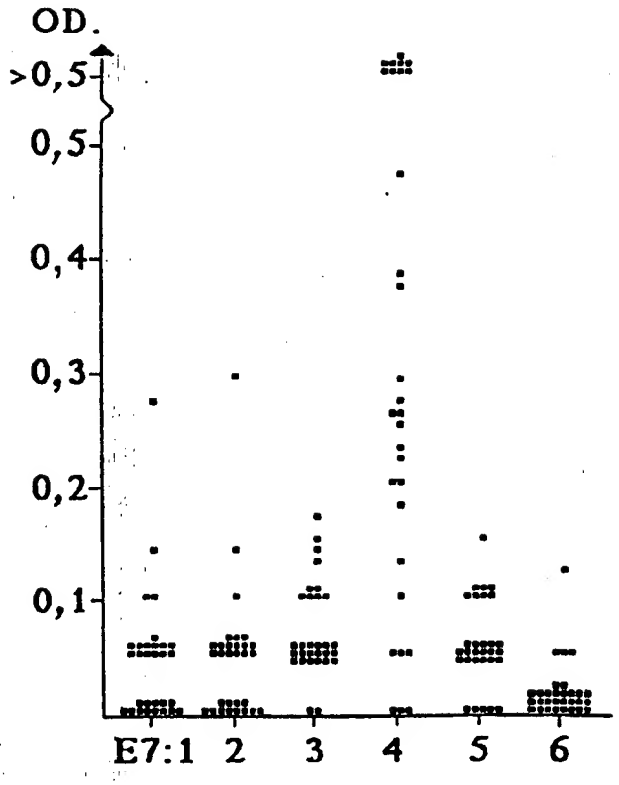
E7, IgA

FIG. 16



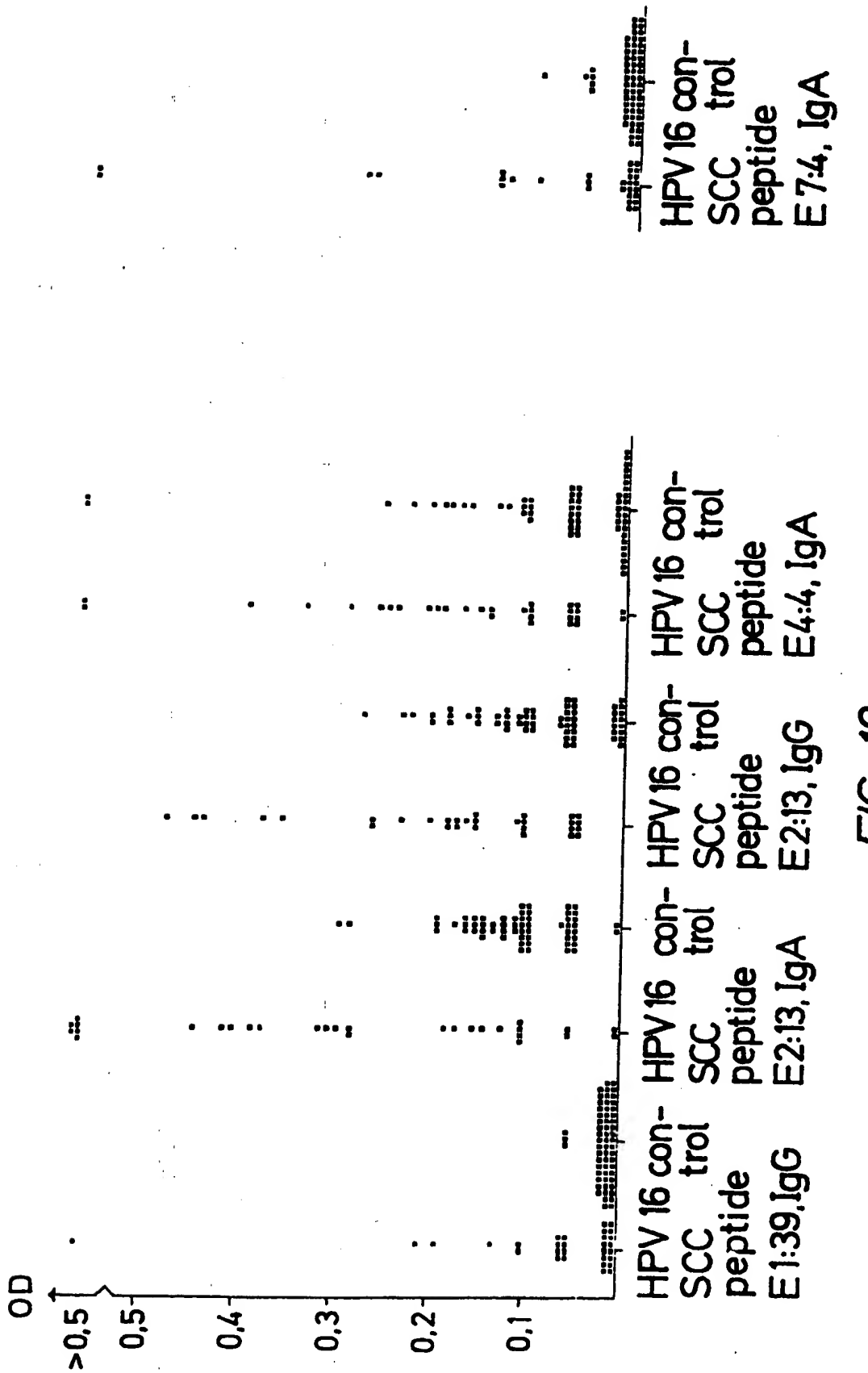
E7, IgG

FIG. 17



E7, IgM

FIG. 18


OD
>0,5
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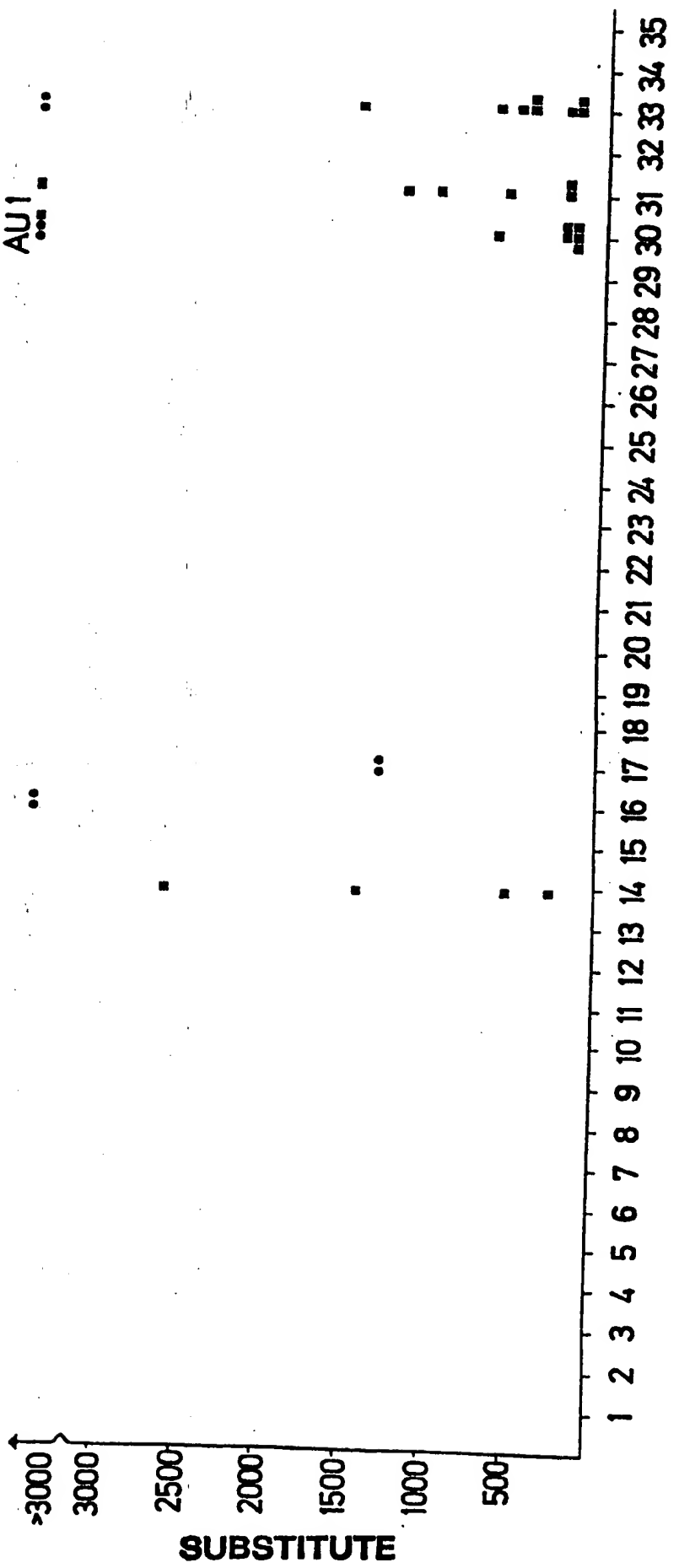
0,4
0,3
0,2
0,1
HPV 16 control SCC peptide E1:39, IgG
HPV 16 control SCC peptide E2:13, IgA
HPV 16 control SCC peptide E2:13, IgG
HPV 16 control SCC peptide E4:4, IgA
HPV 16 control SCC peptide E7:4, IgA

FIG. 19


AU1
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35
>3000
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2500
2000
1500
1000
500
SUBSTITUTE

FIG. 20

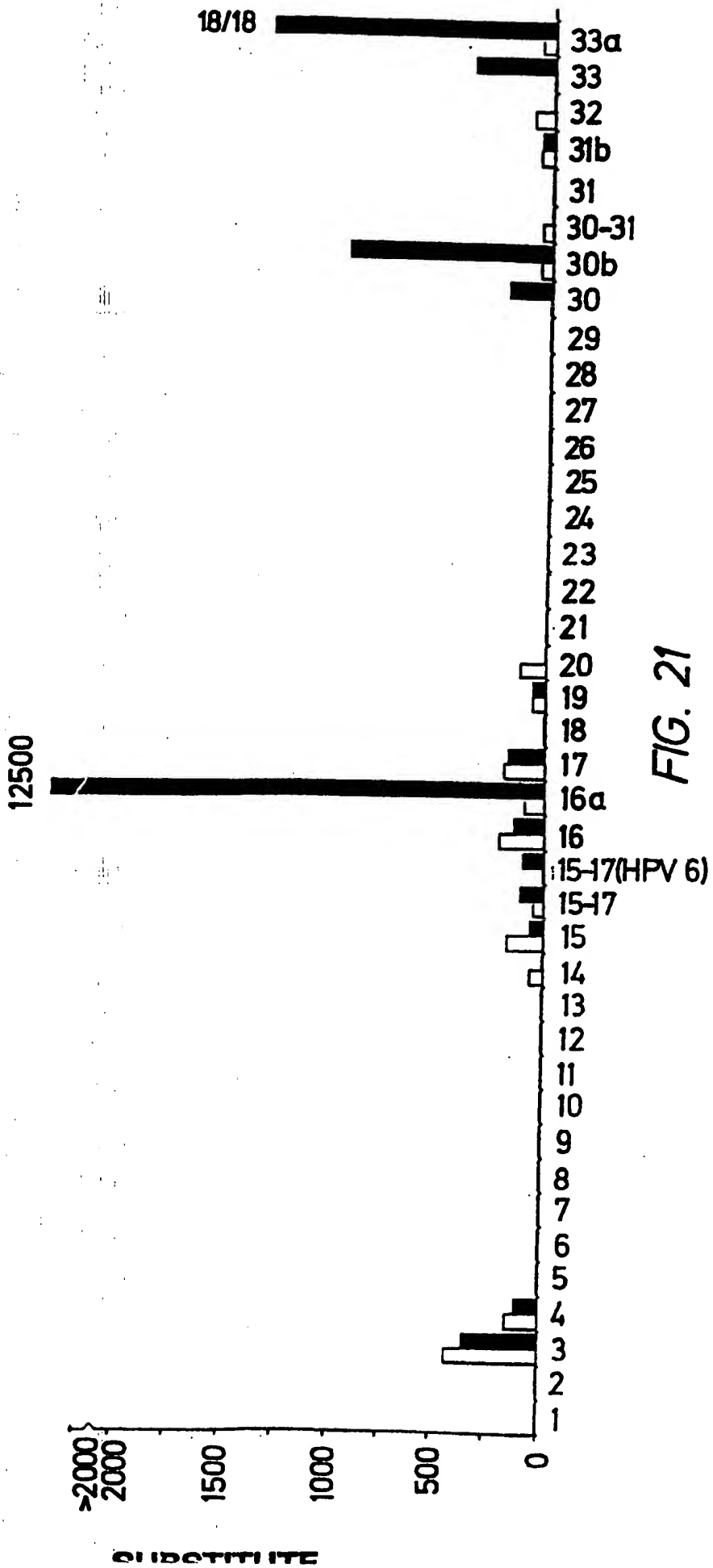

FIG. 21

INTERNATIONAL SEARCH REPORT

International Application No PCT/SE 91/00335

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC5: G 01 N 33/569, C 07 K 7/04

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
IPC5	C 07 K; G 01 N

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in Fields Searched[8]

SE,DK,FI,NO classes as above

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category *	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	Dialog Information Services, Medline, File 154, Accession no. 07256206 , Steele JC et al: " Humoral assays of human sera to disrupted and nondisrupted epitopes of human papillomavirus type 1" & Virology Feb 1990, 174 (2) p388-98 --	1,2,5,6, 8
X	Dialog Information Services, Medline, File 154, Accession no.06062955, Komly CA et al: "The L2 open reading frame of human papillomavirus type 1a encodes a minor structural protein carrying type-specific antigens" & J Virol (UNITED STATES) Nov 1986, 60 (2) p 813-6 --	1,2
A	Dialog Information Services, Medline, File 154, Accession no. 06638652, Doorbar J et al: " Analysis of HPV-1 E4 gene expression using epitope-defined antibodies" & EMBO J Mar 1988, 7 (3) p825-33 --	1

* Special categories of cited documents:[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance, the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance, the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
15th October 1991	1991 -10- 30
International Searching Authority	**Signature of Authorized Officer**
SWEDISH PATENT OFFICE	Carl Olf Gustafsson

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	EP, A1, 0092456 (INSTITUT PASTEUR) 26 October 1983, see the whole document --	1,2
Y	Proc. Natl. Acad. Sci., Vol. 86, May 1989 Joakim Dillner et al.: "A synthetic peptide defines a serologic IgA response to a human papillomavirus-encoded nuclear antigen expressed in virus-carrying cervical neoplasia", see page 3838, left column and "DISCUSSION" --	1,2,5-11
Y	EP, A2, 0344940 (SCRIPPS CLINIC AND RESEARCH FOUNDATION) 6 December 1989, see in particular pages 33-48 and claims --	1-11
X	WO, A1, 9004790 (MEDSCAND AB) 3 May 1990, See in particular page 29, peptides 16, 30, 31 and claims	1-11
Y	--	1-11
Y	Dialog Information Services, Database WPIL, File 351, Accession no. 5195697, TOA NENRYO KOGYO KK: "Antibody formed to antigen polypeptide - contains hydrophilic part forming part of initial protein of human papilloma-virus relating to malignant diseases; EXAMINATION", & JP 1061665 A 890308 8916 (Basic) --	1,3
X	WO, A1, 8701375 (INSTITUT PASTEUR) 12 March 1987, see page 31 - page 36; page 46 - page 47 --	1,2,5-11
X	WO, A1, 8705630 (INSTITUT PASTEUR) 24 September 1987, see page 14 - page 16; claim 6och7 --	1,2
X	EP, A1, 0256321 (BEHRINGWERKE AKTIENGESELLSCHAFT) 24 February 1988, see page 4 - page 6; claims 3-8 --	1,2,5-11

III. DOCUMENTS CONSIDERED T BE RELEVANT (CONTINUED FR M THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	EP, A2, 0299354 (BEHRINGWERKE AKTIENGESELLSCHAFT) 18 January 1989, see the whole document	1-11
X	EP, A2, 0257754 (THE BOARD OF TRUSTEES OF THE LELAND STANFORD JUNIOR UNIVERSITY) 2 March 1988, See particular page 3, peptides 7, 8, 13 and 14	1-11
Y	J. gen. Virol., Vol. 70, 1989 D Patel et al.: "Reactivities of Polyclonal and Monoclonal Antibodies Raised to the Major Capsid Protein of Human Papillomavirus Type 16", see page 69 - page 77 See in particular Table 1 and pages 76-77	1,2,5-11
Y	Virology, Vol. 175, 1990 N D Christensen et al.: "Immunological Cross-Reactivity to Laboratory-Produced HPV-11Virions of Polysera Raised against Bacterially Derived Fusion Proteins and Synthetic Peptides of HPV-6b and HPV-16 Capsid Proteins", see page 1 - page 9 See in particular Table I and "Discussion"	1-11
X	The EMBO Journal, Vol. 6, No. 1, 1987 K Seedorf et al.: "Identification of early proteins of the human papilloma viruses type 16 (HPV 16) and type 18 (HPV 18) in cervical carcinoma cells", see page 139 - page 144 See in particular fig 3	1,2
Y		1-11
X	Int. J. Cancer, Vol. 45, 1990 J Dillner et al.: "MAPPING OF LINEAR EPITOPES OF HUMAN PAPILLOMAVIRUS TYPE 16: THE L1 AND L2 OPEN READING FRAMES", see page 529 - page 535 See table 1, peptides 39 and 49	1-3,5-11

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
X	J. gen. Virol., Vol. 68, 1987 L Banks et al.: "Expressi n of Human Papillomavirus Type 6 and 16 Capsid Proteins in Bacteria and Their Antigenic Characterization", see page 3081 - page 3089 See page 3085, last paragraph - page 3088, last paragraph	1,2,5-11
X	Virology, Vol. 164, 1988 J M Firzlaff et al.: "Detection of Human Papillomavirus Capsid Antigens in VariousSquamous Epithelial Lesions Using Antibodies Directed against the L1 and L2 Open Reading Frames", see page 467 - page 477	1,2,8-10
Y	National Library of Medline (NLM), Database Medline, accession no.90107938, Barbosa MS et al: "The region of the HPV E7 oncoprotein homologous to adenovirus E1a and Sv40 large T antigen contains separate domains for Rb binding and casein kinase II phosphorylation", & EMBO J 1990 Jan; 9(1):153-60	1
Y	National Library of Medline (NLM), Database Medline, accession no. 90171929, Strang G et al: "Human T cell responses to human papillomavirus type 16 L1 and E6 synthetic peptides: identification of T cell determinants, HLA-DR restriction and virus type specificity", & J Gen Virol 1990 Feb; 71 (Pt 2):423-31	1
A	J. gen. Virol., Vol. 67, 1986 G Matlashewski et al.: "The Expression of Human Papillomavirus Type 18 E6 Protein in Bacteriaand the Produktion of Anti-E6 Antibodies", see page 1909 - page 1916	1,2,5-11
A	J. Gen. Virol., Vol. 69, 1988 H M Browne et al.: "Analysis of the L1 Gene Product of Human Papillomavirus Type 16 by Expression in a Vaccinia Virus Recombinant", see page 1263 - page 1273	1

III. DOCUMENTS C NSIDERED TO BE RELEVANT (C NTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
P,X	EP, A2, 0386734 (BEHRINGWERKE AKTIENGESELLSCHAFT) 12 September 1990, See claims --	1
P,X	EP, A1, 0375555 (MEDGENIX GROUP, S.A.) 27 June 1990, See claims --	1
P,X	EP, A2, 0412762 (MERCK & CO. INC.) 13 February 1991, See claims --	1

FURTHER INFORMATI N C NTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers....., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers....., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

see next sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

1-11 partially. Search for HPV5 and HPV8 ORF protein or peptide based assays omitted.

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the the claims. It is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additonal fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional seach fees.

Use of each group of type specific HPV proteins and most groups of corresponding peptides as well as each peptide from HPV 16, 18 and 6, in immunoassays represents separate units of invention a posteriori (due to the state of the art eg. as revealed in the description).

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.PCT/SE 91/00335

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the Swedish Patent Office EDP file on 30/08/91
The Swedish Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

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